UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

A SHORT REVIEW OF THE CHEMICAL IMMOBILIZATION PRINCIPLES IN SOME COMMON AFRICAN WILDLIFE SPECIES

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“Do you know why is it called veterinary practice?
Because you never stop practicing it”

- **Dr. B. Tindall, 2015**

ACKNOWLEDGEMENTS

To Dr Brendan Tindall, my supervisor, for all the years of knowledge, sarcastic humor and unforgettable wild adventures in South Africa that I will always remember. You are an inspiration for everyone who has goals as huge as mine. Thank you for believing in me and in my ambitions from the beginning, for sharing the same passion that I have for wildlife and for all the good stories you told me in the last years, usually around the fire of a *braai* and under a sky full of stars. You gave me wings to fly.

Thank you to the entire team of *Robberg Veterinary Clinic* in Plettenberg Bay, who allow me to be part of the family during the 5 months I spent there, particularly to Kerry who never left me without work to do; Nix and Viv for always having a smile during chaos and, of course Yvonne 'Pink Lady' Funk for the friendship, the laugh, the x-rays and the coffee. Also to Dr Stephan Nell, for teaching me more than I ever expected. Your faith in my skills made me believe that my life aims are feasible and I can dare to dream even bigger. *Baie dankie!*

Thank you *Wild Spirit*, because without your existence, my dream would be just that – a dream – and it would not have become real. Thank you Mark Rutherfoord, owner of *Gondwana Game Reserve*, Ian Olivier and all the wildlife rangers who provide me some of the best moments of my life along with Brendan and Stephan, performing surgery in your wild lions. I will never forget that day!

To Dr William Fowlds and Dr Peter Caldwell for teaching me the best of Conservation Medicine in South Africa and to Dr Jorge Soares, a mentor and a friend over the past years, for all the memorable captures performed in Mafra.

To Chester Zoo, especially to Dr Steve Unwin, Ian Ashpole and Javier Lopez for the opportunity and the knowledge about Zoological Medicine.

Thank you Prof Dr Sandra Jesus for the full support and advice during the growing process of this thesis.

To my family, not only in Portugal but also in Canada. *Obrigada a todos, em particular à minha mãe e ao meu avô, que acompanhou de perto as minhas aventuras em África, Índia, Canadá e Europa, sempre com histórias suas para contar algures no mundo, levando-me a sonhar com as mais distantes terras e as mais exóticas culturas do planeta.* To Guilherme, for all the constant jokes about my work and for sharing the same wanderlust disease I have.

Carolina, Diana and Lília for craziness! Wild 'Faunas' for passion and ambition.

To my "zoological" family of Lisbon Zoo, particularly to Miguel Lajas, Rita Ramos, Ana Poças, Ana Lemos, Inês Guerra and Marta Reis. You all always gave me the support wherever I was in the world. Thank you for sharing the same level of nerding that I have for wildlife and conservation, and for never let me give up of my goals.

And last but not the least, my thanks to a challenging hardcore cat with a very special wild attitude.

RESUMO

A medicina veterinária associada à vida selvagem têm vindo a evoluir nas últimas décadas graças às constantes mudanças nas técnicas de imobilização, equipamentos e até fármacos utilizados. Estes progressos, aliados à experiência dos veterinários, têm vindo a contribuir para uma prática médica mais segura tanto para os animais como para as equipas envolvidas nos procedimentos.

Durante o estágio na África do Sul, os protocolos anestésicos usados nas imobilizações químicas (184 dos 245 indivíduos imobilizados) foram analisados para as diferentes espécies manipuladas, tendo em conta o equipamento de disparo utilizado, o ambiente envolvente e o propósito das intervenções praticadas. As diferentes imobilizações químicas foram classificadas como bem-sucedidas (176) ou não (8), tendo sido referidas as principais complicações que afectaram os procedimentos. Nos casos sem o sucesso anestésico esperado, recorrendo a um segundo dardo (13 casos) ou culminando na morte dos individuos (3 casos), as razões para o insucesso foram discutidas e algumas medidas preventivas para o futuro foram propostas.

É importante que este tipo de informação seja sempre analisado após a execução de uma imobilização, divulgando as conclusões dessa análise e respectivas experiências pessoais dos casos, de modo a poderem ser exploradas pelos médicos-veterinários de vida selvagem para evitar complicações futuras.

Palavras-chave: Anestesia, Medicina de Vida Selvagem, Princípios de Imobilização Química, Medicina de Conservação, África do Sul.

ABSTRACT

Due to constant changes in restraint techniques, equipment and even immobilization drugs, wildlife veterinary practices have improved over the past decades. This broad progress coupled with the experience of practitioners contributes towards a safer practice for both the animals and people involved in the procedures.

Anesthetic protocols used for chemical immobilizations performed during an internship in South Africa (184 of 245 restrained individuals) were analyzed for the various species approached, taking into account the darting equipment involved, the surrounding environment and some of the main purposes of each intervention. The different chemical immobilizations performed were classified as successful (176) or unsuccessful (8), and the most common complications that affected the procedures were documented. The reasons for the failings that in cases led to a second darting (13 cases) or to the death of animals (3 cases) are also addressed and preventive measures to avoid them were put forward.

It is important to analyze the information logged after every immobilization procedure, including personal experiences from each clinical case, and present and disseminate the conclusions, to help wildlife practitioners worldwide avoid future complications.

Keywords: Anesthesia, Wildlife Practice, Chemical Immobilization Principles, Conservation Medicine, South Africa.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
½	Half
¼	One quarter
¾	Three quarters
x	Times
+	Plus
’’	Inches
^{o}C	Celsius degrees
&	And
α	Alpha
μ	Mu (Receptors)
δ	Delta (Receptors)
κ	Kappa (Receptors)
®	Registered Trade-mark
ACTH	Adrenocorticotropic Hormone
NSAIDs	Non-steroidal anti-inflammatory drugs
ANS	Autonomic Nervous System
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
cc	Cubic centimeter
CM	Capture Myopathy
CNS	Central Nervous System
CO_2	Carbon dioxide
CR	*Critically Endangered*
CRT	Capillary Refill Time
CK	Creatinine Phosphokinase
DD	*Data Deficient*
DNA	Deoxyribonucleic Acid
DOP	Delta Opioid Peptide
Dr	Doctor
e.g.	*Exempli gratia*
EN	*Endangered*
EX	*Extinct*
EW	*Extinct in the Wild*
FMD	Foot and Mouth Disease

g	Gram
GC	Gel Collar
GIT	Gastrointestinal Tract
GPS	Global Positioning System
GnRH	Gonadotropin-Releasing Hormone
HPA	Hypothalamic-pituitary-adrenal
IM	Intramuscular
IU	International Units
IUCN	International Union for Conservation of Nature
IV	Intravenous
Kg	Kilogram
KOP	Kappa Opioid Peptide
L	Liter
LAN	Long-Acting Neuroleptics
LC	*Least Concern*
LDH	Lactate Dehydrogenase
m	Meter
mg	Milligram
mL	Milliliter
mm	Millimeter
MOP	Mu Opioid Peptide
NE	*Not Evaluated*
NMDA	N-Metil-D-Aspartate
NT	*Near Threatened*
PNS	Peripheral Nervous System
Prof	Professor
SA	South Africa
SECONDS	Species, Environment, Capture related factors, Other diseases, Nutrition, Drugs, Signalment
SNS	Sympathetic Nervous System
SSC	Species Survival Commission
SQ	Subcutaneous
TB	Bovine Tuberculosis
TZ	Tiletamine/Zolazepam
US	Ultra-sound
VU	*Vulnerable*
WB	Wire Barb

CHAPTER 1.
INTRODUCTION

1.1 INTERNSHIP TRAINING ACTIVITIES

This academic thesis aims to provide a short review of immobilization principles in the wild by documenting examples of clinical cases based on data gathered during an internship in South Africa (SA). The training period (over 1100 hours) between July 27th and December 21st of 2015 was supervised by Dr Brendan Tindall of the Robberg Veterinary Clinic in Plettenberg Bay. The vast majority of the training consisted of improving practical skills in wildlife and conservation, and applying knowledge mostly acquired on previous South African wildlife courses undertaken in recent years. However, different fields of veterinary medicine, such as zoological medicine, exotic pets, equines, small and farm animals also made up part of the work carried out.

SA is one of the countries where the practice of veterinary medicine in the wildlife field is more advanced. National parks, private reserves and game farms teaming with

wildlife allow the veterinarians to develop and improve techniques used to immobilize free-ranging animals. The veterinary practitioners involved in these procedures require a particular knowledge and experience due to the existing biodiversity of species and the variety of conditions to which the animals are exposed. Also, it is important to bear in mind that most of the wild species usually subject to veterinary procedures have an important conservational value, which raises the responsibility of the veterinarian.

Table 1 shows the different species immobilized during the internship period and their classification according to the IUCN (International Union for Conservation of Nature) Red List of Threatened Species. This list has several categories and criteria to classify the species according to their relative risk of extinction. All species are categorized though some are classified as *Not Evaluated (NE)* or even as *Data Deficient* – DD (absence of adequate data). When the information available is reliable, the species can be properly classified as: *Least Concern* (LC), *Near Threatened* (NT), *Vulnerable* (VU), *Endangered* (EN), *and Critically Endangered* (CR) – these last three categories being for threatened species - with the final stages being: *Extinct in the Wild* (EW) and *Extinct* (EX). (http://www.iucnredlist.org)

Table 1 – List of the species immobilized during the internship and their classification by IUCN Red List of Threatened Species (Bauer, Packer, Funston, Henschel & Nowell, 2015; Blanc, 2008; Durant, Mitchell, Ipavec & Groom, 2015; Emslie, 2012; Fennessy & Brown, 2010; Hack & Lorenzen, 2008; Henschel et al., 2008; IUCN SSC Antelope Specialist Group, 2008a; IUCN SSC Antelope Specialist Group, 2008b; IUCN SSC Antelope Specialist Group, 2008c; IUCN SSC Antelope Specialist Group, 2008d; IUCN SSC Antelope Specialist Group, 2008e; IUCN SSC Antelope Specialist Group, 2008f; IUCN SSC Antelope Specialist Group, 2008g; IUCN SSC Antelope Specialist Group, 2008h; Orrell, 2016; Woodroffe & Sillero-Zubiri, 2012; IUCN/SSC Cat Specialist Group, 2015).

SPECIES		**IUCN RED LIST**
African Buffalo	*Syncerus caffer,* Sparrman 1779	Least Concern
African Elephant	*Loxodonta africana,* Blumenbach 1797	Vulnerable
African Lion	*Panthera leo,* Linnaeus 1758	Vulnerable
Blue Duiker	*Philantomba monticola,* Thunberg 1789	Least Concern
Blue Wildebeest (Golden)	*Connochaetes taurinus,* Burchell 1823	Least Concern
Bontebok	*Damaliscus pygargus pygargus,* Pallas 1767	Near Threatened
Bushbuck	*Tragelaphus scriptus,* Pallas 1766	Least Concern
Cheetah	*Acinonyx jubatus,* Schreber 1776	Vulnerable
Leopard	*Panthera pardus,* Linnaeus 1758	Near Threatened
Gemsbok (Golden)	*Oryx gazella,* Linnaeus 1758	Least Concern
Giraffe	*Giraffa camelopardalis,* Linnaeus 1758	Least Concern

Plains Zebra	*Equus quagga,* Boddaert 1785	Least Concern
Roan Antelope	*Hippotragus equinus,* Saint-Hilaire 1803	Least Concern
Sable Antelope	*Hippotragus niger, Harris 1838*	Least Concern
Springbok	*Antidorcas marsupialis,* Zimmermann 1780	Least Concern
African Wild Dog	*Lycaon pictus,* Temminck 1820	Endangered
White Rhinoceros	*Ceratotherium simum*, Burchell 1817	Near Threatened

Wildlife practice is a very distinctive and specialized area of veterinary medicine. Restraining an individual might require the use of anesthetic drugs to allow a safe and efficient immobilization before the practitioner proceeds with plans for the targeted individual. Anesthesia plays an important role in wildlife veterinary medicine as the majority of the procedures carried out in SA involved chemical restraint techniques.

Preventive medicine, surgery, imagiology and, obviously, anesthesia were the key areas of the interventions carried out on the wildlife. Each time an individual was immobilized various procedures were performed on the animal to take advantage of the immobilization. For example, a sable antelope, immobilized for re-location purposes, was also bio-measured, ear-tagged, implanted with a microchip, vaccinated and de-wormed. In certain instances blood and hair collection was also performed for deoxyribonucleic acid (DNA) testing.

This thesis aims to present chemical immobilization principles and methods used by the practitioners in free-ranging African species, comparing the different approaches, protocols, doses and reversals observed during the internship.

CHAPTER 2.

LITERATURE REVIEW

2.1 ANESTHESIOLOGY IN WILDLIFE

Wildlife veterinary medicine is closely associated with anesthesiology because of the non-domesticated nature of the animals and the vast majority of interventions require the anesthetic immobilization of the individuals (Hernandez, 2014; Foggin, Masterson & Hoare, 2012; Thurmon & Short, 2007).

Originating from the Greek *anaisthaesia*, the word ‘anesthesia’ means total or partial ‘insensibility’ of the body, absence of sensations (Clarke, Trim & Hall, 2014). Most wildlife examinations are usually performed under general anesthesia to provide a safe procedure for the animal, the veterinarian and the personnel involved (Thurmon & Short, 2007). *Analgesia*, also a Greek term, is used to describe the absence of pain (Clarke et al., 2014). This status can sometimes be mistaken for tranquilization (when the animal presents a change in its behavior, becoming more relaxed and less anxious – but yet aware – without

drowsiness) or sedation (depression and drowsiness – not aware). Local anesthesia or analgesia is a loss of painful sensation in a specific area of the body (but yet the animal is still apparently aware) while regional analgesia is a loss in a larger area. General anesthesia induces a depression of the central nervous system (CNS) (Clarke et al., 2014; Thurmon & Short, 2007).

Clinical pharmacology – the study of drugs and respective interaction in the organism – in wildlife practice can sometimes be neglected due to the difficulties in working with non-domesticated animals. This oversight can increase the risk of secondary effects in the individual and jeopardize the safety of the immobilization for the animal and the personnel (Clarke et al., 2014; Lamont & Grimm, 2014; Thurmon & Short, 2007). For this reason, limiting stress conditions and improving monitoring and clinical care can be advantageous (Clarke et al., 2014; Lamont & Grimm, 2014). The drug effect in the organism is intentional but it can have adverse effects. The relationship between the dose and the effects is called pharmacodynamics (*What the Drug Does to the Body*, Clarke et al., 2014) and it is different for each individual, as is the route of administration, its absorption, the distribution to the tissues, biotransformation and elimination from the body (pharmacokinetics: description of the processes associated with the drug's movement – *What the Body Does to the Drug*, Clarke et al., 2014) (Clarke et al., 2014; Lamont & Grimm, 2014; Thurmon & Short, 2007).

The ideal immobilizing drug for wildlife interventions must have the following important characteristics:

- Versatility to be used in different species,
- High potency in a small volume,
- Wide margin of safety,
- Quick action,
- Reversible effects,
- Rapid elimination from the body,
- Minimal side effects,
- Calm induction and recovery,
- Minimal handling risk,
- Stability,
- Analgesic properties (Foggin et al., 2012; Muir, 2007; Thurmon & Short, 2007).

2.2 IMMOBILIZATION TECHNIQUES

Restraint techniques to immobilize wildlife are many and varied. The different methods can be divided in two different restraint groups: physical restraint techniques and chemical restraint techniques. Each one is better suited to particular species or situations (Hernandez, 2014; Atkinson, Kock & Meltzer, 2012; Shury, 2014). Most of the techniques

used in free-ranging animals were extrapolated in the late 20th century from zoos, parks and farms. Restraint techniques have become an ingenious art thanks to the experience of the practitioners, as well as becoming safer for the animals and the people involved (Shury, 2014).

2.2.1 PHYSICAL RESTRAINT

The physical restraint of a free-ranging animal must follow a series of basic principles that are also applied in zoo medicine: it must be safe for the people involved in the procedure and for the animals; mortality rate and injuries should be minimal. The technique must be simple and easily executed by non-experienced practitioners. Equipment must be portable and easy to set up, and the procedure has to be swift enough to allow the animal to return to its normal physiological state (Hernandez, 2014; Shury, 2014). It should also be usable in combination with chemical restraint to improve the immobilization and the efficiency of the performance (Atkinson et al., 2012). Other factors such as cost, season, time of the day, species, behavior, sex and age of the animal, number of people involved and environmental conditions must also be considered (Hernandez, 2014; Shury, 2014; Atkinson et al., 2012; La Grange, 2012).

Free-ranging animals can be physically restrained individually, using box/cage traps (passive capture) and net guns (using a helicopter), or en-masse by using mass capture techniques like bomas or nets (drop net, rocket net, drive net) (Hernandez, 2014; Shury, 2014; La Grange, 2012).

a) Physical force

Using physical restraint to immobilize wildlife is simple but can only be used on limited species such as small mammals, birds and reptiles (Atkinson et al., 2012).

b) Passive capture

Animals are caught and trapped in an enclosure – box, cage, crate – using water or bait to lure them. The enclosure entrance is closed manually or automatically once the animal is inside. It is a selective method, relatively stress-free for the animal and turns out low mortality rates. It is reusable and requires minimum staff. However, it can be very time consuming (Hernandez, 2014; Shury, 2014; La Grange, 2012).

c) Bomas

Bomas are one of the oldest and most-used techniques in mass captures for ungulates, especially in re-location procedures and for testing and research purposes (Shury, 2014, Atkinson et al., 2012). A physical and visual barrier is used to drive the animals into a desired direction, usually into a vehicle (Atkinson et al., 2012). The principle of this technique is simple: the herd of animals is driven into a funnel-shaped enclosure guided by a helicopter while a series of curtains gradually draw to behind the animals as they move forward. The final curtain corrals the herd and forces the animals directly into a transport truck using a ramp (Figure 1). Once inside the truck, the herd is usually pole-syringed with long-acting neuroleptics (LANs). Another option is to dart the herd in the final enclosure, load it by hand and reverse the animals once inside (this second option is mostly performed on aggressive and valuable animals like sable and roan antelope). This method has low mortality rates and allows for a large number of animals to be caught in a short period with very little handling. On the down side, this technique involves expensive equipment, is labor intensive and requires experience. It is also weather dependent and time consuming (Fivaz & Ebedes, 2012; La Grange, 2012).

Figure 1 – Boma used to capture a herd of zebras in 2012 (Original).

d) Nets

Animals usually need to be driven into the nets (net-boma) by the practitioners on foot, using horses, vehicles or aircraft like helicopters or fixed wings (Shury, 2014). Once this has been achieved the nets collapse and entangle the animals. They are hand-restrained and loaded with or without immobilization and/or tranquilization. This method is appropriate for certain species (e. g. nyala - *Tragelaphus angasii*, or bushbuck) that inhabit thick vegetation and often difficult terrain. The equipment is cost-efficient, easy to set up and is less weather dependent than the boma. Nevertheless, it is labor-intensive and requires an experienced team to manage the animals that can easily be injured and stressed. This

procedure can also be hazardous to the staff (Goodman, Hedley & Meredith, 2013; La Grange, 2012, Fivaz & Ebedes, 2012; Bothma & Van Rooyen, 2005).

e) Net-Gun

A more recent technique, the net-gun, was developed in the 1970s in New Zealand to capture red deer (*Cervus elaphus*). It soon spread to North America where it was used to capture ungulates without using immobilizing drugs, although the technique can be combined with chemical immobilization to extend the restraint in certain situations (Shury, 2014; Jacques et al., 2009). In cases where chemical immobilization cannot be performed this particular gun is limited by the species to be captured and environmental conditions. (Walzer & Gerritsmann, 2015; Goodman et al., 2013).

2.2.2 CHEMICAL RESTRAINT

Wildlife medicine veterinarians need to administer anesthetic drugs effectively using different types of remote delivery systems (Hernandez, 2014; Arnemo, Evans, Fahlman & Caulkett, 2014; Isaza, 2014). The practitioner selects the best system for each situation according to the behavior and cooperation of the animal (Isaza, 2014; Atkinson et al., 2012). The procedure chosen by the veterinarian for trained animals in zoos (controlled environment) will differ from the procedure chosen for a free-ranging unpredictable animal (Hernandez, 2014; Isaza, 2014, Fahlman, 2008).

Chemical restraint is used mainly for large ungulates and carnivores when physical restraint is impractical. This chemical method is not usually appropriate for immobilizing large numbers of animals but it is the primary choice if an individual requires medical assistance. It is expensive and its success could depend on the experience of the veterinarian in charge (Hernandez, 2014).

There are different approaches to administering different drugs in a chemical restraint procedure: oral, hand-held injection, pole syringe, and using darts (Hernandez, 2014; Isaza, 2014). In cooperative animals, hand-held injections or the pole-syringed administration are the delivery routes of choice. If the animal is uncooperative, remote delivery systems using blow darts, gunpowder explosive darts or compressed gas projectors are the most suitable choice for the practitioner (Isaza, 2014).

a) Oral

Oral administration is inevitably dependent on the ability to ensure the animal accepts the drug. For this reason, its absorption and effect make it an unreliable method. Oral administration is used mainly in carnivores or primates for sedation before darting and requires high doses of immobilization drugs. In ruminants, because of slow absorption rates

and large mass of ingesta, this method is not commonly used (Atkinson et al., 2012; Burroughs, Meltzer & Morkel, 2012a).

b) Hand-held Injection

This administration route is only used in animals that are already physically restrained or cooperative in behavior. The practitioner must administer an effective rapid intramuscular (IM) injection but must be careful to prevent accidental self-injection. The drug is pulled into a plastic syringe with a specific needle attached (Isaza, 2014; Goodman et al., 2013; Atkinson et al., 2012).

c) Pole Syringe

This method is used as an extension of the hand and it allows for the injection of drugs in animals already in enclosures (crates, transport vehicles from the roof-top) or even trees (Hernandez, 2014; Goodman et al., 2013; Kock, Jessup & Burroughs, 2012a). It follows the same principles as the hand-held injection but has a long pole attached to the syringe, providing a safer procedure than the hand-held option. The drug is pulled into the syringe, which usually has a lower gauge needle. The syringe is embedded in the pole and it is ready to use (Hernandez, 2014; Isaza, 2014; Atkinson et al., 2012). The veterinarian must approach the animal as quietly as possible and then inject it, fast and forcefully, through the skin into the muscle. It should always be borne in mind that the animal could still react and cause injuries and it is therefore advisable for the injector to wait until he has the best shot possible, because if a first attempt is missed the animal can become agitated, making it more difficult to obtain a nice and clear second attempt (Kock et al., 2012a).

d) Blow Dart (Blow Pipe)

As its effectiveness is limited to short distances (15-25 meters [m], being more accurate at 8-10m) the blow dart is mainly used in controlled environments like zoos, bomas, trees or crates (Hernandez, 2014; Goodman et al., 2013; Atkinson et al., 2012; Kock et al., 2012a) This system has a silent projection and a lower dart impact energy which results in a softer impact to the animal, preventing possible injuries caused by the dart. It can be used on small and large animals by blowing in one firm exhalation to propel the dart, which requires a lot of practice (Hernandez, 2014; Atkinson et al., 2012).

e) Darts

The most common technique used for a chemical restraint in free-ranging animals is darts fired from dart guns. The darts are very versatile and offer many varied remote delivery systems, giving the practitioner a choice of different equipment to be used according to the situation (darts can be used in bomas, zoos, from vehicles, from helicopters,

from horses or even by foot) from a distance of between 2m and 100m (Isaza, 2014; Goodman et al., 2013; Atkinson et al., 2012; Kock, Jessup & Burroughs, 2012b). The first semblance of a dart was developed in 1958, and the authors described it as a 'flying syringe' that used an acid-base reaction to administer the drug(s) into the animal. Due to its reliability and efficiency the remote delivery system remained unchanged for the 55 years subsequent to its development (Walzer & Gerritsmann, 2015).

For maximum accuracy, dart guns require an experienced practitioner who is familiar with the equipment, skilled at estimating the distance of the shot (or in using a rangefinder), and able to calibrate the charge according to that distance (Walzer & Gerritsmann, 2015; Hernandez, 2014).

Those adjustments need to be made quickly and quietly before the animal moves, avoiding excessive impact to prevent accidents such as tissue damage, bone fractures or body cavity penetrations in the darted animal (Walzer & Gerritsmann, 2015; Hernandez, 2014; Arnemo et al., 2014; Atkinson et al., 2012). Aside a proper knowledge of wild animals' anatomy, physiology and their behavior, the practitioner must also have enough field experience to avoid the dangerous pitfalls associated with wildlife work (the danger of working with carnivores is, normally, well judged but ungulates can also be potentially lethal due to bites, kicks and injuries caused by the horns) (Hernandez, 2014).

Impact energy is the amount of energy generated when the dart strikes the animal – ½ dart mass x velocity^2. A dart, ideally with the minimum drug volume in the smallest dart possible, traces an arc through the air and drops over a distance, hitting the animal, and hopefully providing a good IM injection (Atkinson et al., 2012). Darts can be use in animals that otherwise would be impossible to immobilize and are less stressful for them than the physical capture. Darts have low mortality rates and are safer for the personnel involved. However, it is a time consuming exercise (only a few animals at a time can be targeted) that is also expensive, and requires a very experienced veterinarian (Isaza, 2014; Atkinson et al., 2012).

A dart can be divided in four components: the drug(s) storage chamber, the discharge method, the needle required to

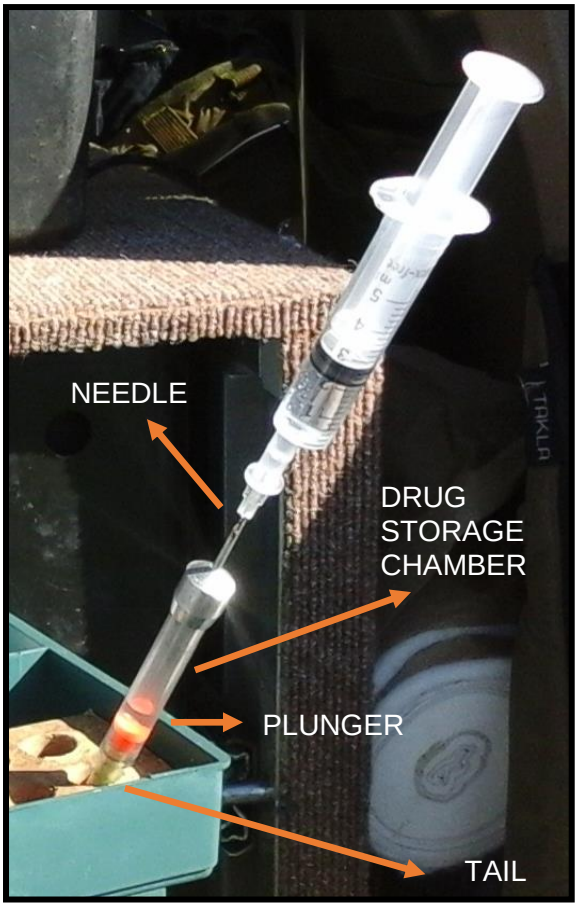


Figure 2 - *Pneu-Dart*® dart projectile Components (Original).

penetrate the animal and a stabilizer for balance during the flight (Isaza, 2014). Figure 2 represents the different components of a *Pneu-Dart*® (Pneu-Dart, Inc., Philadelphia, USA) dart projectile with a stabilizer colored tail.

Transmitter darts are a specific type of dart mainly used in situations where the vegetation is dense (for nyala or a bushbuck), with the potential of the darted animal getting lost. They can be supplied by *Tele-Dart*® (Tele-Dart, Westheim, Germany), *Cap-Chur*® (Palmer Cap-Chur Equipment Inc., Georgia, USA), *Pneu-Dart*® and *Dan-Inject*® (Dan-Inject Equipment, Borkop, Denmark) (Kock et al., 2012b).

2.3 TYPES OF DARTS AND DART GUNS

The chemical immobilization of wildlife usually requires remote drug delivery systems (Hernandez, 2014). Due to the difficulties in approaching a free-ranging wild animal, an effective and reliable remote injection system that is able to hit the animal over long distances without causing unnecessary complications or injuries is required. The performance of different systems is evaluated by their range and accuracy (Walzer & Gerritsmann, 2015; Hernandez, 2014). These remote delivery systems consist of a dart and a projector, which can be a blowpipe (blow dart), a compressed air projector or a gunpowder cartridge rifle (powder charges) (Hernandez, 2014; Isaza, 2014; Kock et al., 2012b; Kock, Jessup & Morkel, 2012c).

2.3.1 TWO-CHAMBERED COMPRESSED GAS DARTS (PRESSURIZED)

Two-chambered compressed gas darts (pressurized) are usually made of plastic, with the two chambers being divided by a plunger. The front chamber is for the drug solution and the back chamber for pressurized gas (Hernandez, 2014). Firstly, before the practitioner even starts to fill up the dart, the system must be tested by pressurizing and depressurizing the dart. Only then it is ready for the veterinarian to load the drug(s) into the anterior chamber. The needle is attached, taking care to cover the needle's side hole with a silicone sleeve (using a *Leatherman*® [Leatherman Tool Group Inc., Oregon, USA] to make the needle tightly attached). Then, given that the needle's side-point is covered with a sleeve, the operator can pressurize the posterior chamber with compressed gas/air. This is introduced into the chamber manually by using an adapter attached to a syringe (usually a 20 ml syringe) (Hernandez, 2014; Isaza, 2014; Kock et al., 2012b). Finally, the stabilizer – the tailpiece – is attached to the back of the dart. When the dart hits the skin of the animal, the silicone sleeve on the needle slides down allowing the drug to be injected into the animal (Isaza, 2014). The pressurized gas moves the plunger forward and pushes the drug(s) into the anterior chamber, and out through the needle hole (Hernandez, 2014). These darts are lightweight, quiet and less traumatic for the tissues thanks to a slower injection (Hernandez,

2014; Isaza, 2014). By choosing the right needle-lengths, gauges and characteristics, this type of dart can be used in almost every species. They are usually used for short to medium distances with clear trajectories, in good weather conditions, from the ground or air, and particularly in zoos or controlled environments (Isaza, 2014; Kock et al., 2012b). As these darts are usually transparent, the veterinarian can verify that the entire volume of the dart was injected (which make it safer when removed). However, compressed gas darts are not recommended for all species (e.g. they are not advised for carnivores as they can easily break the plastic dart with their teeth). *Telinject*® (Telinject Remote Injection Equipment, California, USA) and *Dan-Inject*® are the major manufacturers of these types of darts/guns offering a large variety of darts (volume, needle sizes, tail pieces) that can be reused after proper cleaning and storage (Hernandez, 2014; Isaza, 2014). As the darts are reusable there is a risk that they might fail after repeated use due to pressurizing problems or damage of the plastic (Kock et al., 2012b).

The pressurized systems and non-pressurized explosive systems are both very reliable, but with the former, the veterinarian requires experience and practice to guarantee a correct pressurization of the dart (Kock et al., 2012b).

2.3.2 GUNPOWDER EXPLOSIVE DARTS

Gunpowder Explosive Darts have a gunpowder cap as a mechanism to discharge the drug(s). A plunger is inserted to divide the anterior chamber with the drug(s) solution from the posterior chamber with the explosive cap, a spring and a weighted firing pin. A tailpiece is attached to the back of the dart as a stabilizer (Hernandez, 2014; Isaza, 2014). To prepare this type of dart, the practitioner has to fill the anterior chamber with the drug(s) he is planning to use, through the needle. Needles can vary greatly in length, gauge and characteristics. When the dart hits the animal the firing pin collides with the cap, which results in detonation. The expanding gas moves the plunger forward and the drug(s) inject into the animal through the needle. This is a faster mechanism in comparison to the pressurized darts with greater chances of causing muscle trauma, particularly in small species (Hernandez, 2014; Isaza, 2014; Kock et al., 2012b). This method can use modular gunpowder explosive-powered reusable darts (e.g. *Palmer Cap-Chur*®) – one of the oldest models and the first efficient dart manufactured in the 1960s – or prefabricated gunpowder explosive-powered disposable darts (*Pneu-Dart*®) (Hernandez, 2014; Kock et al., 2012b).

The practitioner should be particularly careful when using Cap-Chur® darts as if the charge is accidentally attached backwards it will detonate on firing and spray the drugs out of the dart gun barrel and some of the drugs commonly used in wildlife can be lethal to humans (Kock et al., 2012b).

The other disadvantages of these darts are the cost and the opaque plastic or aluminum material of the darts, which prevents the practitioner from seeing if the plunger has

moved forward (Hernandez, 2014). The main difference between them is that with the prefabricated dart model, the dart is a complete unit that cannot be dismantled. The explosive mechanism has to be replaced by the company and cannot be reused (Hernandez, 2014; Isaza, 2014; Kock et al., 2012b). *Pneu-Dart*® has two different types of darts: a 'P' type with a yellow tail (and no ridge on the tail) on the back for blowpipes, carbon dioxide (CO_2) powered projectors or air-pumped guns; and the type 'C' with an orange tail on the back (larger than the yellow of type 'P' and with a ridge) for .22 blank-powered cartridge projectors (Isaza, 2014; Kock et al., 2012b). In both types, the veterinarian has to select the appropriate dart for the procedure, according to the species, choosing the length, gauge and characteristics of the needles (Isaza, 2014). The dart must be inspected and loaded with the drug(s). If the drug(s) does not occupy the total volume of the chamber, the practitioner must top up the dart with sterile water or 0.9% sodium chloride (NaCl) solution to avoid a loss of balance during the trajectory (Hernandez, 2014; Isaza, 2014). The tailpieces attached to the back of the darts are colored to make it easier to find if the dart gets lost on the ground (Kock et al., 2012b).

Besides the darts mentioned, there are more types, associated with a large variety of different dart guns: the Aluminum Two-Chambered Compressed Gas Darts for *Palmer Cap-Chur®,* the Chemical-Powered Darts (with and acid-base reaction which results in a gas production to expel the dart's content), the Spring-Powered Darts or even Solid Drug Darts (Isaza, 2014).

Additionally to the type of dart or remote delivery system used, it is always good practice to mark the syringes during the procedures (Kock et al., 2012b).

2.3.3 REMOTE DELIVERY SYSTEMS

Usually used in controlled environments such as zoos or on confined animals, blowpipes and gauged blowgun projectors are two of the most common compressed gas projectors that allow a short distance shot. The blowgun system is very similar to the blowpipe but it has an external compressed gas source, eliminating the need for the practitioner to exhale through the pipe. One of the most commonly-used blowguns is manufactured by *Dan-Inject*® (Isaza, 2014).

Air or CO_2 rifles and pistols are commonly used in free-ranging wildlife. The compressed gas such as CO_2, comes in cylinders or in air pumps and the veterinarian has to select the amount of gas to use each time he wants to dart an animal. *Palmer Cap-Chur*® and *Pneu-Dart*® type 'P' are the leading manufactures of these projectors. These darts allow for very accurate long-distance shots. However, the dart can cause a significant impact on the animal with traumatic consequences (Isaza, 2014; Kock et al., 2012c).

The *Pneu-Dart*® type 'P' uses a CO_2 cartridge or a reusable CO_2 cylinder and the practitioner can easily control the amount of gas he is using according to the distance

between him and the animal. The latest model of *Pneu-Dart*® type 'P' is simple, durable and relatively inexpensive, allowing the user to dart accurately, quietly and quickly. The weight of this heavy gun can be a problem in the field. However, the *Dan-Inject*® model, also available with CO_2 cylinders, is lighter and yet robust; the downside to this gun is the unreliability on long distance shots (optimal range between 10-40m) or when the veterinarian is darting from a helicopter (Kock et al., 2012c).

Gunpowder cartridge-powered rifles are supplied with .22 caliber blanks of different strengths worked according to color. They are highly accurate and can be used by the practitioner over long distances even in windy conditions. The most common manufacturers are *Palmer Cap-Chur*® and *Pneu-Dart*® type 'C' (Isaza, 2014).

The *Pneu-Dart*® type 'C' models are supplied with powder charges of different strengths, represented by a different color. Brown charges are the lightest, followed by green, yellow and finally red, the strongest one. After loading the charges in the gun extra distance control can be achieved by altering the port adapter in the dart discharge system (Kock et al., 2012c).

Additional dart equipment includes laser range finders, scopes, sights and binoculars. These are vital in calculating the appropriate pressure and ensuring accurate dart placement (Isaza, 2014; Kock et al., 2012c). The sights are particularly useful when darting from the ground in thick bush areas or from a helicopter, whereas telescopic sights are used most in accurate long-distance shots. Some practitioners prefer the red dot point scope, where both eyes are kept open during darting (which is advantageous in situations where dangerous animals are in the area) for both short and long distances, either darting from a helicopter or even at night (commonly when carnivores are being baited). A laser sight can also be an option for highly accurate night darting (Kock et al., 2012c).

Regardless the type of remote darting equipment used, the practitioner must always be familiar with the equipment and practice with it. Experience and practice are the key to a good immobilization procedure (Kock et al., 2012b).

2.4 DARTING SITES

Large muscle masses are the ideal darting sites to allow a correct IM administration. The hindquarters are, in most of the species, the best place to target. However, because of adipose tissue, in some species the base of the neck or the triceps muscle are more suitable (Hernandez, 2014).

- Base of the neck region: This area is suitable for animals with heavy necks such as the rhino, the hippopotamus (*Hippopotamus amphibious*) or large antelopes like the kudu (*Tragelaphus strepsiceros)* causing little muscle trauma. The veterinarian must be aware that the dart could strike a cervical vertebra, vital blood vessels or the nuchal ligament. It can also

be quite irritating for the animal (Hernandez, 2014; Arnemo et al., 2014; Burroughs et al., 2012b).

- Triceps muscle region: This is a suitable area for most species. In thin animals there is a risk of the dart striking the scapula cartilage, the scapula spine or vital blood vessels in the neck. The biceps and triceps are easy to dart from the ground, particularly in animals like the rhino or the elephant. If the animal is too thin, its hindquarters are the preferable area for darting (Hernandez, 2014; Atkinson et al., 2012).

- Hindquarters: With the most muscle mass for a large target area, the hindquarters is the most common and desirable darting site (Hernandez, 2014). It has only a few vital structures (although care must be taken to avoid penetrating the femur and pelvis if the animal is struck on the medial side of the hind leg) and it can be accessed either from the ground or a helicopter. The veterinarian must dart the animal perpendicularly to the surface to ensure a deep IM injection. If the practitioner is darting the animal from behind, he must take care to avoid the soft tissue area of the perineum, which can cause problems, particularly in equines (Atkinson et al., 2012).

- Withers and rump: These spots can be a small target when the practitioner aims from the side or from above (using a helicopter) but usually represent a good muscle mass, especially in the eland - *Taurotragus oryx* and the rhino (Atkinson et al., 2012; McTaggart, Kock & Hofmeyr, 2012).

- Chest: Due to the risk of hitting the head or penetrating the thoracic cavity, causing pneumothorax, the chest is not a common place to dart an animal. The pectoral area is an option for darting if no alternative is available. Rhino, buffalo, giraffe or eland are suitable for pectoral darting (Atkinson et al., 2012; Kock, La Grange & du Toit, 1990).

The following figures (3,4,5,6,7 and 8) represent the different darting sites in some of the most common African wildlife species.

Figure 3 and 4 – Best darting sites in some of the most common African large ungulates (Adapted from Kock & Burroughs, 2012).

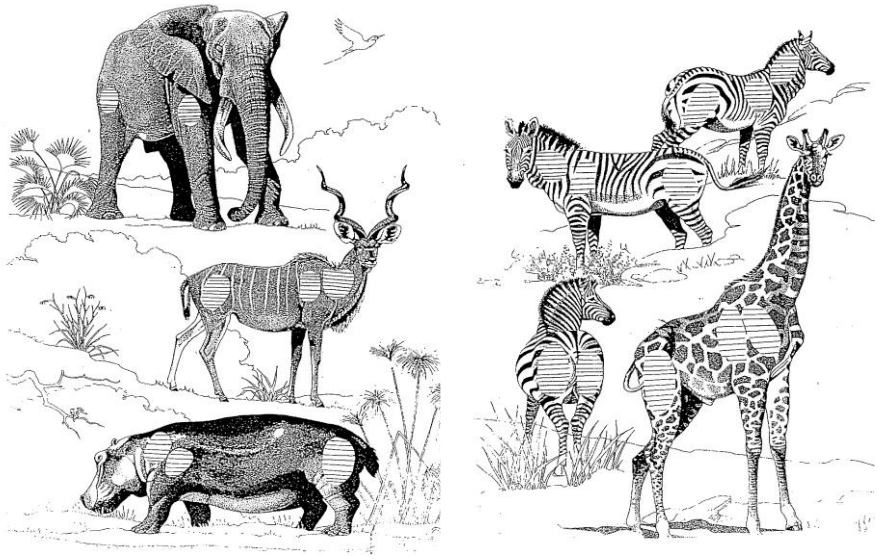


Figure 5 and 6 – Best darting sites in some of the most common African wildlife species (Adapted from Kock & Burroughs, 2012).

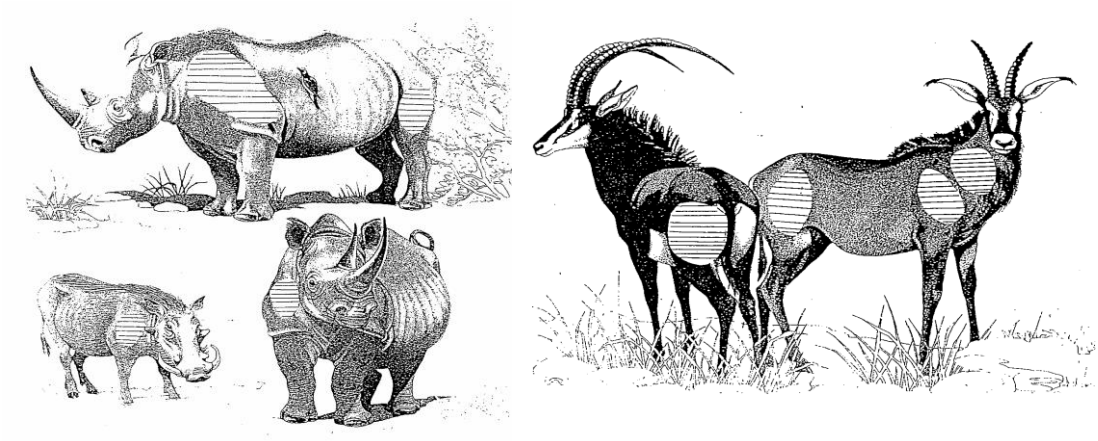
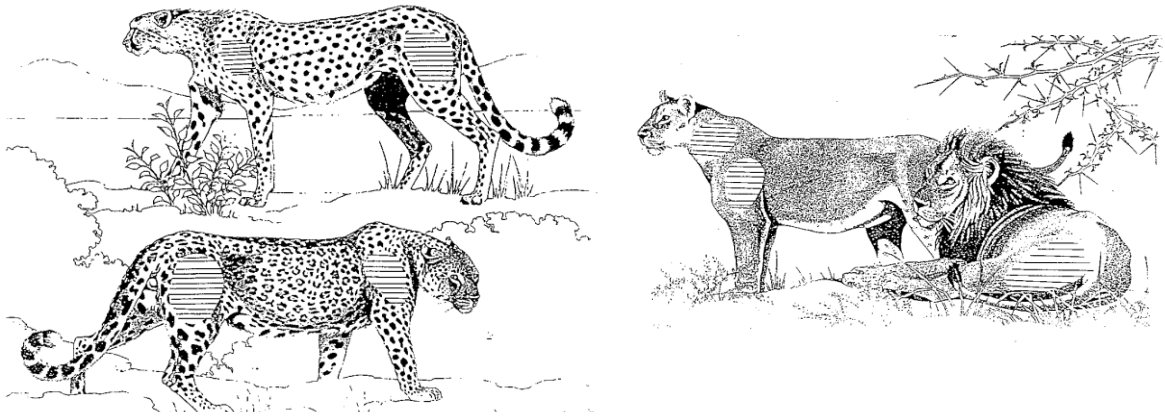


Figure 7 and 8 – Best darting sites in some of the most common African wild carnivores (Adapted from Kock & Burroughs, 2012).



2.5 NEEDLE LENGHTS, GAUGES AND CHARACTERISTICS

There are numerous types of darts, each one with a specific volume (cubic centimeter, cc), needle length (inches, ''), needle gauge and with characteristics that match their purpose. The length of a needle is very important to a successful immobilization and avoiding unnecessary injury or pain to the animal. The practitioner also needs to bear in mind the gauge of the needle and characteristics such as collars or barbs (Hernandez, 2014; Atkinson et al., 2012). However, the practitioner's decision will also be influenced by the choice of drug(s). If the dart is to be filled with antibiotics (which have a high viscosity), a lower gauge needle is required (Atkinson et al., 2012).

The length of the needle is usually associated with the size of the animal to be darted, the thickness of the skin and the depth of the muscles. For an effective darting the drug(s) must be injected in the deep muscles, which requires an accurate dart placement (Hernandez, 2014). Logically, longer needles will provide a better dart attachment within the animal and, if the practitioner wants to make sure that the dart is going to stay firmly embedded in the animal, he can opt to use barbed needles. A barbed dart can stay attached to the animal long enough for the practitioner to recover it while the animal is immobilized. However, the barb can cause more muscle damage than plain or collared darts. The practitioner has to extract the barbs correctly to avoid further trauma (Atkinson et al., 2012). These types of darts can be used in antelopes, buffalos or rhinos. A drop-out dart, with collared needles, preferably the gelatinous absorbable type, is usually used by the practitioner when darting an animal for vaccination (Atkinson et al., 2012).

Table 19 (Appendix 1.) shows the best dart characteristics according to the information collected from the clinical cases during the internship and based on the experience of Dr Brendan Tindall (unpublished data).

2.6 FACTORS THAT INFLUENCE A CAPTURE

Most of the free-ranging wildlife in SA has to be immobilized in a capture procedure. Various factors can affect that immobilization: species, environment, capture-related factors, other diseases, nutritional status, drugs used to immobilize the animal and administration route, and signalment. The veterinarian in charge must evaluate these seven categories of predisposing factors with the mnemonic SECONDS on every procedure (Arnemo et al., 2014; Hernandez, 2014; Shury, 2014; Atkinson et al., 2012; La Grange, 2012).

Different species have different responses to the same drug and/or dose. Tamed individuals or individuals in a controlled environment may have a better response to a lower dose than free-ranging animals (Atkinson et al., 2012). Environmental factors (e.g. humidity, high temperatures, terrain) are also an important consideration. During the induction phase animals in areas inaccessible to vehicles can become lost or injured (e.g. falling from a cliff

or drowning) and should not be immobilized (Arnemo et al., 2014; Ko & Krimins, 2014; Shury, 2014; Atkinson et al., 2012). Weather conditions (e.g. wind, temperature and time of day) must also be considered when planning an immobilization (Arnemo et al., 2014; Hernandez, 2014; Shury, 2014). Crosswinds might change the dart's trajectory; procedures during the late afternoon could result in an animal becoming lost in the dark; high temperatures could cause hyperthermia in animals, and low temperatures could result in hypothermia, which can both be fatal. The habitat must also be evaluated. Areas where the bush is too thick might be a problem to dart and find the animal (Arnemo et al., 2014; Hernandez, 2014; Ko & Krimins, 2014; Atkinson et al., 2012; Meltzer & Kock, 2012). Capture-related factors such as the techniques, handling methods, transportation, position of the animal or injuries during the procedure can play a role, as well as other diseases (underlying infectious diseases, parasites, anemia or organ damage), which can predispose the individual to clinical problems during the immobilization. The nutritional status of the animals can be related to dehydration, pre-existent vitamin E and selenium deficiency or other minerals and vitamins or poor body condition (e.g. season related). These different conditions can interfere with the planned anesthetic protocol. The drugs used depend on the species to immobilize and it falls to the veterinarian to choose the most adequate combination suitable for the situation. An opioid combination with tranquilizers or sedatives is usually used in ungulates, while in carnivores it is preferable to use combinations of cyclohexylamine and sedatives. These combinations have different effects on each species and each individual (Burroughs et al., 2012a; Foggin et al., 2012). The route of administration must follow manufacturers' pharmacological instructions and recommendations to improve the immobilization and avoid problems (e. g. drugs formulated with oils being administered intravenously) (Atkinson et al., 2012; Burroughs et al., 2012b). When the veterinarian uses a pole syringe or dart to immobilize the animal, the best route of administration is IM, although in cases the dart or syringe can embed subcutaneously. The best IM administration sites are in the hindquarters, shoulder or neck and are dependent on the species and the line of sight. Depending on the drug of choice the intravenous (IV) route can be preferable for topping up an animal that is starting to wake from anesthesia, as well as for the administration of a fast reversal drug (Atkinson et al., 2012; Burroughs et al., 2012a; Burroughs et al., 2012b; Kock et al., 2012a). Finally, signalment has to be taken into account (e.g. sex – some studies affirm that males have a higher risk of developing severe complications such as capture myopathy, and estrogens have a protective effect in females; age; pregnancy) (Blumstein et al., 2015; Wolfe, 2015; Hernandez, 2014; Pas, 2014; Paterson, 2014; Hofmeyr, Fivaz & Meltzer, 2012; Atkinson et al., 2012; Sanchez, 2011).

2.7 PHASES OF IMMOBILIZATION

To avoid complications, the practitioner must follow this sequence of events: Planning and preparation phase, Approach phase, Induction phase, Handling and monitoring phase, Reversal/Recovery phase and the Reflection phase (Arnemo et al, 2014; Shury, 2014; Atkinson et al., 2012).

2.7.1 PLANNING AND PREPARATION

The main goal of this phase is to organize the actions that the operation requires, according to the purpose of the immobilization. An adequate plan of action must be established and the entire team informed (Arnemo et al., 2014; Hernandez, 2014). The veterinarian in charge must prepare the drugs, the equipment and give instructions to the personnel, including human safety issues to avoid major complications during the immobilization. He has an ethical responsibility towards the animal and the choice of what is best must be elaborated also in accordance with the animal's behavior and its health status/body condition, always bearing in mind the specific individual's sensitivity (Hernandez, 2014; Atkinson et al., 2012; La Grange, 2012).

As previously mentioned, the terrain is an important factor in capturing free-ranging species. The practitioner or a member of the team must be familiar with the terrain and its conditions. Flat or open areas might lead the veterinarian to plan a slower induction whereas thick wooded areas must be associated with a faster induction or the use of telemetry/drones to avoid losing the animal. The same can occur if the weather is excessively hot and humid, which can force the procedure to be rescheduled for the benefit of the animal. The veterinarian must be familiar with the drugs available and abide by the principle that it is preferable to overdose than to underdose (Arnemo et al., 2014; Ko & Krimins, 2014; Burroughs et al., 2012a; La Grange, 2012).

A crucial part of the planning phase is the choice of equipment (Hernandez, 2014). Besides the drugs' delivery systems, drugs and eventual medication, blindfolds, earplugs, water, ropes, stretchers and other equipment must be readily available during the immobilization procedure (Arnemo et al., 2014; Goodman et al., 2013; Atkinson et al., 2012; Kock, 2012).

2.7.2 APPROACH

The approach and the darting are the two most important actions during the immobilization process. Although a successful approach and a successful darting improve the procedure, its success is not guaranteed (Atkinson et al., 2012). This phase is more important in free-ranging wildlife than in zoo immobilizations as some of the wild species can become excited and run, sometimes for long distances before the intended effect of the drugs kicks in (Arnemo et al., 2014; Atkinson et al., 2012). Whenever possible the

veterinarian should avoid darting on foot or using vehicles that the animals are not familiar with. He should stay on the road; avoid disturbing the bushes or changing the shape of the vehicles well as using the minimum people required. Patience is essential and chasing animals will increase their stress levels as well as the difficulty of the approach and the darting (Atkinson et al., 2012). The darting must be done when the animal is not moving, at a maximum range of 40-50m (except if the darting is taking place from an helicopter, where the ideal scenario is to have the helicopter and the animal moving at the same speed) (Atkinson et al., 2012; McTaggart et al., 2012). If there is a high risk of losing the animal, the use of a helicopter could be the best choice for the darting. Helicopters are a very efficient form of approach, particularly if the terrain does not allow a ground approach (Figure 9); although transmitter darts might be an option if no helicopter is available (Atkinson et al., 2012; Kock et al., 2012b; McTaggart et al., 2012). The best approach after the darting is to leave the animal undisturbed, if possible, otherwise, if it runs, a chase will be necessary to keep it in sight (Atkinson et al., 2012).

Figure 9 – Helicopter chasing a white rhino in an inaccessible area to improve the success of darting. The aim of the procedure was to trim the rhino's horn due to poaching atempts in the reserve in 2014 (Original).

2.7.3 INDUCTION

The induction time is defined as the time between the injection/darting and the effective immobilization of the animal (Atkinson et al., 2012). The veterinarian has to be aware of the variable factors (drugs, specie, stress, dart, dosage) that can influence the induction phase, which usually takes between 3-15 minutes (Hernandez, 2014). A shorter induction time might indicate that the animal was overdosed, whereas a longer induction time can suggest an underdosage and the animal might require extra immobilization drugs (always bear in mind that *it is better go higher with the dose,* especially with opioids – Burroughs et al., 2012a). First, the animal will usually experience a stage of alarm, followed by a wariness, then disorientation/agitation and possible behavior changes, mainly in locomotion such as running and ataxia. This could cause the animal to stumble or move

away from the herd, possibly towards vehicles or trees, and loss of balance. An inexperienced veterinarian might find it harder to notice such changes. This phase is followed by a phase of excitement ("hackney gait") and then, if the quantity of the dose suffices, the animal loses coordination and falls into recumbency. If the dosage is insufficient the animal will take longer to reach recumbency, and might in fact never reach this stage, experiencing instead a prolonged stage of excitement which increases the risk of injury and hyperthermia, exhaustion and, consequently, can lead to death (Ko & Krimins, 2014; Radcliffe & Morkel, 2014; Atkinson et al., 2012). The veterinarian must be particularly aware of the animals' recumbency as for example, ruminants normally have to be in a sternal position to avoid bloat, and species like carnivores must have their eyes covered with blindfolds (Arnemo et al., 2014; Hernandez, 2014). Additionally, the practitioner and the team involved must be acquainted with the terrain and able to anticipate the behavior of the animal and the potential risks (Hernandez, 2014). The use of vehicles and helicopters to guide the animal to a safe area and monitoring it with binoculars without getting close might be an option (Arnemo et al., 2014; Hernandez, 2014; Ozeki & Caulkett, 2014; McTaggart et al., 2012).

2.7.4 HANDLING AND MONITORING

Anesthesia affects all animals differently: they can be found on their feet but handleable; lying down, although they still get up when approached; down with spontaneous movement of the head; down with no voluntary movement (caution); down with poor breathing (danger).

The veterinarian must take care when approaching the animal and should move it into a correct position if necessary (e.g. herbivores in a sternal position with the nose down; elephants in a lateral position) as well as evaluate the depth of the anesthesia. If necessary he should cover the animals' eyes with blindfolds and use earplugs to block the noise. Carnivores may need their eyes lubricated before being blindfolded (Hernandez, 2014; Atkinson et al., 2012; Hofmeyr et al., 2012). Monitoring is essential and basic references such as body temperature, respiratory rate, heart rate, and capillary refill time (CRF) should be recorded at least every 5-10 minutes. Major anesthetic complications can be anticipated by clinical signs such as depression, weakness, abnormal body temperature, cold extremities, abnormal heart rate and/or respiratory rate, cyanotic mucous membranes, slow capillary refill time, tremors, neurological signs, pain, and lameness. A pulse oximeter might also be a useful tool to have in the field (Hernandez, 2014; Ozeki & Caulkett, 2014; Atkinson et al., 2012; Hofmeyr et al., 2012). At the same time, the dart must be collected and safely discarded to avoid accidents. The dart wound must be inspected and treated for trauma injuries (Hernandez, 2014; Atkinson et al., 2012). Some of the most common complications that can occur during wildlife capture procedures will be explained in detail later on (Hernandez, 2014; Ozeki & Caulkett, 2014). The veterinarian can also administer: wound

treatment to the dart injury (e.g. penicillin), other antibiotics, vitamins, anti-inflammatory drugs, eye ointments, analgesics, cleaning solutions and, if necessary, initiate emergency/reversal (or partial reversal) drugs (Atkinson et al., 2012; Hofmeyr et al., 2012; Kock et al., 2012a). If necessary, the team must be prepared to cool the animal down to avoid any possible consequences of hyperthermia, and if the temperature does not go down despite that cooling, the practitioner might need to administer antidotes to antagonize the effects of the anesthesia (Hernandez, 2014; Ko & Krimins, 2014).

2.7.5 REVERSAL/RECOVERY

The recovery of an animal will depend on the procedure, the anesthetic protocol and the species. The reversal administered by the veterinarian might be a total or a partial antagonist of the main drug(s) used (Arnemo et al., 2014; Hernandez, 2014). For example, Ketamine is a dissociative that cannot be reversed but, because it is usually combined with α-2-agonists like Medetomidine, the practitioner can use an antidote to reverse the sedative (Hernandez, 2014; Burroughs et al., 2012a). A partial reversal might be necessary to load the animal in some cases, or to improve safety conditions for the people involved, who must be distanced from the animal when the practitioner completely reverses the anesthesia (Hernandez, 2014; Atkinson et al., 2012). The reversal(s) should be given intravenously, but in some cases, such as with carnivores, an IM injection can be a better option. Then the blindfold and earplugs must be removed and all the equipment must be relocated to a safe area (Arnemo et al., 2014; Hernandez, 2014; Atkinson et al., 2012; Burroughs, Hofmeyr et al., 2012b). The recovery environment has to be assessed: to ensure a calm recovery, the spot cannot be exposed to sunlight, to wind or other animals, and, if possible, the practitioner must observe the animal from a safe distance until it is fully recovered, able to walk, protect itself from other animals, and avoid hazards (Arnemo et al., 2014; Hernandez, 2014).

2.7.6 REFLECTION

After every immobilization procedure the practitioner should take some ‘reflection’ time to log all the doses and drugs used, the methods he decided to use, any mistakes made and any improvements he could adopt in future immobilization processes. Some practitioners use data capture sheets to ensure all details are recorded properly, mainly for research purposes (Goodman et al., 2013).

2.8 WILDLIFE PRACTICE APPLIED PHARMACOLOGY

2.8.1 INTRODUCTION

Different wildlife species have different reactions to the restraint method chosen by the practitioner (Hernandez, 2014; Fahlman, 2008). Consequently, these methods have to be adapted to the species and its behavior in order to cause as little stress as possible and

minimal physiological disturbance, safeguarding the welfare of the immobilized animal (Hernandez, 2014; Fowler, 1995). Most of the procedures in wildlife practice require the individuals to be chemically immobilized, which in itself represents challenges and risks for the practitioner involved (Hernandez, 2014; Schumacher, 2008). Over the years the drugs available on the market have improved, allowing the practitioners to adopt chemical restraint methods over physical, or in association with the latter (Swan, 1993). Unfortunately, there is no perfect drug available with all the characteristics of an ideal anesthetic and the veterinarian has to choose the best drug(s) according to the situation with which he is faced with (Muir, 2007). For example, the drug of choice for one particular species might be inappropriate for another. Opioids, frequently used to immobilize ungulates, can cause several problems, such as respiratory depression in primates, or excitement in felids (Fahlman, 2008). The practitioner has to be familiar with the characteristics of every drug available and be versed in using it, not forgetting that free-ranging animals usually require higher doses than individuals in a controlled environment, as mentioned earlier (Atkinson et al., 2012; Fahlman, 2008; Muir, 2007). Initially, in the earliest chemical immobilizations, practitioners took advantage of the properties of the neuromuscular blockers and used it in the restraints by paralyzing the skeletal muscle, but the animals were still aware and conscious, increasing stress levels and resulting in high mortality rates (Caulkett & Arnemo, 2007). Nowadays, thanks to improved knowledge, veterinarians are using drugs that cause total or partial depression of the CNS, combining different drugs with synergetic effects in order to decrease doses and reduce side effects on the animal (Fahlman, 2008; Schumacher, 2008). Because of all the variables involved in free-ranging wildlife practice there is no ideal protocol for immobilization procedures. Nowadays, however, the main aim of these operations is to minimize the morbidity and mortality of the individuals by decreasing the excitement-free induction period, ensuring proper muscle relaxation, adequate analgesia, decreasing the cardiopulmonary depression effects and ending the immobilization with a rapid and smooth recovery after the administration of the reversal drug(s) (Schumacher, 2008).

2.8.2 IMMOBILIZATION AGENTS

2.8.2.1 OPIOIDS

This group of drug is probably the most important in the chemical immobilization of ungulates. Nevertheless, the opioids family also confers analgesic effects on the management of pain in many different species through specific opioid receptors (Lamont & Grimm, 2014; Ramsay, 2008; Schumacher, 2008). There are different types of opioid receptors: mu (μ) receptors – mu opioid peptide (MOP), delta (δ) receptors – delta opioid peptide (DOP) and kappa (κ) receptors – kappa opioid peptide (KOP) (Lamont & Grimm, 2014; Ramsay, 2008). The μ receptors are associated with analgesic properties and some

side effects associated with opioid administration. On the other hand, δ receptors have minor analgesic effects but they are able to modify other receptors; the κ receptors are associated with the analgesia in some specific areas on the central and peripheral nervous system (PNS). Some natural molecules produced by the organism in the CNS, in the adrenal gland or even in the hypophysis are endogenous opioid peptides, which couple in the endogenous opioid receptor and mediate some analgesia. Exogenous systemic administration of opioids (IV, IM or SQ) and endogenous opioids act on the CNS receptors. Opioids can cause depression of the CNS, leading to sedation, or stimulation of the CNS and, consequently, excitement, depending on the species and the dose that is administered (Lamont & Grimm, 2014). Opioids can also affect the thermoregulation center, causing hypothermia (or hyperthermia in some species); the emetic center, by causing nausea and vomiting because of the stimulation of the chemoreceptor trigger zone; they can also cause suppression of the cough center and changes in the pupillary diameter – mydriasis in case of excitement. The respiratory system is also affected by a dose-dependent depression of ventilation (bradypnea), and at a cardiovascular level, bradycardia can occur as well as arterial hypertension (or hypotension in some cases). Ruminants might be predisposed to gastrointestinal complications associated with the administration of opioids (e.g. bloat) or problems often associated with myopathies (Wolfe, 2015; Lamont & Grimm, 2014; Schumacher, 2008). These complications and the undesirable effects are often observed when the opioids are administered alone, without any synergetic drugs associated to allow for a lower dose of the opioid agent and, consequently, fewer side effects on the animal (Schumacher, 2008).

The opioids used in veterinary practice are usually morphine derivatives which react with endorphin receptors such as μ, δ and κ receptors in the brain, spinal cord, autonomic nervous system (ANS), mesenteric plexus of the gastrointestinal tract (GIT), cardiovascular system and kidneys, affecting mainly pain, behavior, and voluntary muscle and GIT motility (Lamont & Grimm, 2014). The most common effects are sedation, excitement, ataxia, analgesia, respiratory depression, hyper or hypotension, hypothermia and inhibition of the GIT motility. Drugs available on the market can act as opioid agonists, antagonists or partial agonists/partial antagonists and they can have a large range of effects on the animal (Lamont & Grimm, 2014; Ramsay, 2008).

2.8.2.1.1 Etorphine hydrochloride

This drug is probably the most widely used immobilization drug for African ungulates, preferably reversed with diprenorphine hydrochloride (Janssen & Allen, 2015; Burroughs et al., 2012a; Schumacher, 2008). Due to its sedative properties etorphine is mainly used to restrain wildlife (it is rarely used as an analgesic) (Lamont & Grimm, 2014). It has a quick onset, starting with a slight ataxia, which progresses to an evident ataxic event,

high stepping gait can be visualized and some of the animals might run while others might stand until falling into a recumbency position – sternal or lateral (Wolfe, 2015; Lamont & Grimm, 2014; Burroughs et al., 2012a). The doses are calculated according to the species not the body mass of the animal (Wolfe, 2015; Burroughs et al., 2012a; Atkinson et al., 2012; La Grange, 2012). To reduce side effects, improve the induction time and avoid hypertonicity it is mainly used in combination with a tranquilizer (e.g. butyrophenone) or a sedative (e.g. α-2-adrenergic-agonists) (Lamont & Grimm, 2014; Burroughs et al., 2012a; Fowler, 2008; Schumacher, 2008). The reversal drug – diprenorphine, naltrexone, naloxone – must be given as quickly as possible to avoid the side effects of the etorphine: respiratory depression, which can lead to severe hypoxia, acidosis and death; cardiovascular changes (initially it starts with hypertension and changes to hypotension); excitement; hypertonicity; hyperthermia; convulsions; GIT stasis, which can result in bloat, regurgitation, aspiration pneumonia (ruminants require a sternal recumbency, head up with the nose down) (Burroughs et al., 2012a; Schumacher, 2008; Caulkett & Arnemo, 2007; Nielsen, 1999). The induction time of Etorphine is longer when compared to other opioid agents. However, the recovery takes about 1-3 minutes after IV injection of the reversal agent (or 5-10 minutes when the practitioner opts for an IM administration) (Schumacher, 2008; Caulkett & Arnemo, 2007).

2.8.2.1.2 Thiafentanil oxalate

Thiafentanil is a synthetic opioid like etorphine (Burroughs et al., 2012a). It has a similar potency and similar properties when compared to carfentanil or even etorphine, but a shorter induction time, allowing a faster immobilization of herbivores, fewer cardiopulmonary depression effects and a shorter half-life (Burroughs et al., 2012a; Schumacher, 2008). When the practitioner chooses to use thiafentanil, the chances of a complication called renarcotization are lower than with etorphine or carfentanil because of the short time of action of these drugs, allowing for a proper anesthetic reversal with an antidote (usually naltrexone) (Burroughs et al., 2012a; Schumacher, 2008; Allen, 1996). It is used in species with a higher sensitivity to etorphine (e.g. sable antelope) or even as a combination of both opioids (e.g. giraffe) (Lamont & Grimm, 2014; Lance & Kenny, 2012; Burroughs et al., 2012a; Burroughs et al., 2012b; Janssen et al., 1991).

2.8.2.1.3 Carfentanil

Carfentanil is no longer used in SA as a registered product but it is still available in the United States of America (USA) (Burroughs et al., 2012a; Caulkett & Arnemo, 2007). It is a derivative of fentanyl and it is more potent than etorphine, with a faster onset of action (induction time around 2-5 minutes) but a longer duration of action (Lamont & Grimm, 2014; Fowler, 2008; Schumacher, 2008). It is not completely reversible with diprenorphine, not

even the side effects on the CNS. Naltrexone is the most efficient reversal, in a ratio dose of carfentanil 90:1 or 100:1 (Lamont & Grimm, 2014; Fowler, 2008; Caulkett & Arnemo, 2007).

2.8.2.2 OPIOID ANTAGONISTS

The biggest advantage of using opioids in wildlife practice lies in its reversible properties with the proper antagonist (Ramsay, 2008; Caulkett & Arnemo, 2007; Nielsen, 1999). Opioid antagonists are used to reverse the effects of opioids by binding with opioid receptors in the body. When administered in high doses, or because they have a better affinity to the receptors, these antagonists compete with the opioids and replace them at the receptors level. Some antagonists can also be used to not completely reverse the animal but, for example, to lighten the immobilization and reduce the respiratory depression or other side effects. They can be divided in two groups: the mixed antagonists, with a slight agonistic effect (diprenorphine, butorphanol, nalbuphine, nalorphine) also known as partial antagonists/agonists or agonist-antagonists; and the pure antagonists (naltrexone, naloxone), depending on the relative concentration between agonistic and antagonistic effects (Lamont & Grimm, 2014; Burroughs et al., 2012a). Partial antagonists compete for the μ receptors, where they have an antagonistic effect, but they act as agonists for the κ receptors. These drugs were developed for acting as analgesics with fewer respiratory depression effects. Contrarily, pure opioid antagonists have an affinity for both μ and κ receptors (Lamont & Grimm, 2014).

2.8.2.2.1 Butorphanol tartrate

This synthetic partial antagonist interacts with the κ receptors as an agonist opioid, inducing analgesic effects, and as an antagonist on the μ receptors, reversing the effect of some of the most potent opioids (Lamont & Grimm, 2014; Burroughs et al., 2012a; Ramsay, 2008). It is commonly administered in combination with other drugs (e.g. azaperone or medetomidine) and its properties allow it to be used in standing sedations, for walking/transporting rhinos and to improve breathing in deep immobilization cases with opioids by manipulating the level of anesthesia (Miller & Buss, 2015; Radcliffe & Morkel, 2014; Burroughs et al., 2012a).

2.8.2.2.2 Diprenorphine

This partial antagonist is the most commonly used antidote for etorphine (can be used to reverse thiafentanil but it is less effective) (Burroughs et al., 2012a). At lower doses, it has antagonistic effects in the animal (e.g. improving the respiration after the IV administration) but in higher doses (ratio of diprenorphine 10:1) or if repeated, it has agonistic effects. The doses are calculated according to the opioid used for the immobilization: 3 times (x) the dose of the etorphine used for large animals or 2x the dose for

the rest of the ungulates, and it can be administered by IV or IM injection (Lamont & Grimm, 2014; Burroughs et al., 2012a).

2.8.2.2.3 Nalbuphine & Nalophine

The characteristics of these two drugs are very similar to butorphanol. Nalbuphine is considered a very similar version of the previous drug used by veterinarians, nalophine (Lamont & Grimm, 2014; Burroughs et al., 2012a). They are used to improve animals' breathing in a deep anesthetic stage and in procedures that require walking rhinos (Burroughs et al., 2012a).

2.8.2.2.4 Naltrexone

Naltrexone is used to antagonize the action of the opioids on the μ receptors. Contrary to the other previously mentioned antagonists, this is a pure antagonist, with a long half-life (no/lower renarcotization risk) and it can be used in human opioid overdoses (Burroughs et al., 2012a). Naltrexone is able to act as an antidote to all the effects of the different opioids (including the renarcotization with carfentanil), improving the animal's response, the perception of pain and awareness. Once administered, the animal cannot be re-immobilized in the next 24 hours (Lamont & Grimm, 2014; Burroughs et al., 2012a; Caulkett & Arnemo, 2007; Allen, 1996). It can be administered by IM or IV injections (Caulkett & Arnemo, 2007). The ratio to be used is 10:1 although some authors refer 20 mg of naltrexone *per* mg of etorphine (Burroughs et al., 2012a) or 100mg *per* mg of carfentanil (Fowler, 2008; Nielsen, 1999). In white rhinos, naltrexone must be always given when the animal is released into the field (Burroughs et al., 2012a).

2.8.2.2.5 Naloxone

This drug is also a pure antagonist and it has been the first choice for human intoxications with opioids (Naltrexone can also be used). Because of its short action, the opioid used might still have an effect on the animal immobilized (Caulkett & Shury, 2014; Morkel & Kock, 2012; Burroughs et al., 2012a). Consequently, animals immobilized with opioids and reversed with naloxone must be monitored to ensure renarcotization does not occur. Naloxone can reverse the effects of all opioids and increase the sense of reaction, awareness, perception and pain (Lamont & Grimm, 2014; Caulkett & Shury, 2014).

2.8.2.3 CYCLOHEXYLAMINES

The cyclohexylamine group is also known as the dissociative anesthetics used as knock-down drugs in wildlife practice. There are no antidotes to reverse the effect of the dissociative agents and usually an animal immobilized with a drug of this category requires a longer recovery. Like opioids, cyclohexylamine can be combined with a tranquilizer or a

sedative (which can be reversible) to attenuate its side effects, such as the hypertonia of the skeletal muscle (Burroughs et al., 2012a; Thurmon & Short, 2007). It has a cataleptic effect, analgesic properties (short duration) and cause immobility, loss of consciousness and amnesia (Lamont & Grimm, 2014; Burroughs et al., 2012a; Nielsen, 1999). Even when knocked-down, the animals still maintain the pharyngeal and laryngeal reflexes, as well as the palpebral and corneal reflexes, the tongue might move and the eyelids remain open (it is advised to protect the cornea from the light with eye drops or ophthalmic gel), spontaneous movements of the animals, mydriasis or nystagmus occur (Burroughs et al., 2012a; Thurmon & Short, 2007). Dissociative agents can be used to immobilize carnivores, primates, reptiles or birds without severe effects on the respiratory or cardiovascular systems, even in high doses (Burroughs et al., 2012a; Caulkett & Arnemo, 2007; Nielsen, 1999). Ketamine and tiletamine are the most common types of cyclohexylamine used in wildlife practice, which can be administered as an IM or an IV injection (Lamont & Grimm, 2014; Burroughs et al., 2012a). The induction takes about 5-10 minutes and some of the side effects may include convulsions (mainly if overdosed and, for that reason, it is advised to combine a muscle relaxant and decrease the dose of the cyclohexylamine), hyper-salivation and hyperthermia (which can be the result of the hypertonicity and convulsions) (Burroughs et al., 2012a; Caulkett & Arnemo, 2007). These drugs have an unpredictable effect on the excitement phase (particularly if the veterinarian uses a lower dose), but the animals do not tend to run or 'hackney gait' like the effect of the opioids on the ungulates. The use of α-2-agonists with the cyclohexylamine might cause vomiting in some animals (Radcliffe & Morkel, 2014; Burroughs et al., 2012a).

2.8.2.3.1 Ketamine

Ketamine can be found in different formulations from a large variety of manufacturers and can be used in a broad spectrum of species, including wildlife. Although its various administration routes, in wildlife practice, Ketamine is usually administered by IM injection (usually painful), orally (as bait in higher doses) or by IV injection with an onset of action between 3-5 minutes (complete immobilization in 5-10 minutes), with a smooth induction (Lamont & Grimm, 2014; Burroughs et al., 2012a). Ketamine inhibits GABA (Gamma-AminoButyric Acid), might suppress serotonin, norepinephrine and dopamine in the CNS and activates the limbic system (Plumb, 2008). The effects are dose-dependent with 2-3 hours of duration and might cause convulsions (reason why ketamine is often combined with other drugs to reduce this effect) and consequently hyperthermia (Burroughs et al., 2012a; Caulkett & Arnemo, 2007). It induces anesthesia, catalepsy and amnesia (an advantage if the animal has to be immobilized more than once), but also increases the heart rate and the blood pressure and suppresses the respiratory rate (Lamont & Grimm, 2014; Plumb, 2008). Although ketamine is mainly used in the immobilization of carnivores, it can be

given to ruminants as a top up drug, usually intravenously (Caulkett & Arnemo, 2007; Nielsen, 1999). It is a safe drug with a margin up to 10x the recommended dose (Swan, 1993). Hallucinations are a side effect in humans but it is difficult to evaluate in animals, although some primates and some felids present some abnormal behaviors and vocalization during recovery (Lamont & Grimm, 2014; Fowler, 2008).

2.8.2.3.2 Tiletamine (/Zolazepam)

Tiletamine is available in combination with zolazepam as an injectable anesthetic with a short induction of 5-8 minutes (Lamont & Grimm, 2014; Ramsay, 2014; Burroughs et al., 2012a; Walzer & Huber, 2002). It induces a dissociative anesthesia similar to the one with ketamine but it has a potency 3-4x higher and it is mainly used in carnivores by IM injection (but also possible IV), that need to be immobilized for 2-4 hours or longer (Lamont & Grimm, 2014; Burroughs et al., 2012a; Plumb, 2008; Walzer & Huber, 2002). It can cause muscular hypertonicity but because of the combination with the zolazepam, it is an unusual event. This combination reduces convulsions, induces muscle relaxation and improves the recovery of the animal (Lamont & Grimm, 2014; Burroughs et al., 2012a; Swan, 1993). However, some of the side effects of Tiletamine might include hyperthermia, cyanosis, vomiting and abnormal vocalization (Nielsen, 1999). Some long-action effects on the CNS, which can last 24-48 hours after administration, might include convulsions, weakness and anorexia (Fowler, 2008). Most of the times the Tiletamine/Zolazepam (TZ) combination is used with an α-2-adrenergic-agonist like medetomidine, which is reversible in felids allowing for a reduction of the dose of the dissociative and, consequently, fewer side-effects (Ramsay, 2014).

2.8.2.4 TRANQUILIZERS

Tranquilizers can be often confused with sedatives because both reduce the motor activity of the animal by acting at the adrenergic receptors level in the CNS and in the PNS (Burroughs et al., 2012a). However, tranquilizers have a more selective action, suppressing the behavior response and have potent effects on the autonomic and the endocrine systems (Lamont & Grimm, 2014). The effects are irreversible and are not dose-dependent, which means that if the practitioner increases the dose of the tranquilizer, the effects are prolonged (including the side effects) but the level of tranquilization does not increase, contrary to sedatives, which have a dose-dependent effect (if the veterinarian administers a higher dose than recommended, the effects will increase as well) (Lamont & Grimm, 2014; Burroughs et al., 2012a). The animal shows signs of reduced fear, anxiety and aggressive behavior (Burroughs et al., 2012a). The 2 main groups of tranquilizers used nowadays are the phenothiazine derivatives, like acepromazine (ACP) and the butyrophenone derivatives (e.g. haloperidol) (Lamont & Grimm, 2014; Burroughs et al.,

2012a). Wildlife practice has taken advantage of both groups by associating them with opioids for immobilizations or as long-acting tranquilizers to benefit the translocation of ungulates by promoting effects that can last hours, days or even weeks. Consequently, stress levels decrease and the welfare of the animals improves by reducing trauma injuries and by upgrading adaptation to new environments (Burroughs et al., 2012a; Caulkett & Arnemo, 2007). The action of the tranquilizers blocks dopamine receptors (located in the cerebral cortex, basal ganglia and limbic system) as well as the α - and β - adrenoceptors, with antagonistic effects. Blood pressure decreases, anorexia and convulsions might occur and even inhibition of the thermoregulatory center, but some anti-emetic properties might be favorable. Usually tranquilizers do not have any analgesic effect and they do not have antidotes, in the event the practitioner should decide to reverse their effect, while sedatives often do (Lamont & Grimm, 2014; Burroughs et al., 2012a).

2.8.2.4.1 Acepromazine

ACP is probably the most used phenothiazine derivative in veterinary practice, combined or not with an opioid – historically named neuroleptanalgesia (Lamont & Grimm, 2014; Montané et al., 2003). In general, phenothiazines block the dopamine receptors at post-synaptic level in the CNS and might inhibit the release of dopamine (Montané et al., 2003). Side effects such as bradycardia, hypotension and a low hematocrit are often present. ACP has also an antiemetic effect, reduces the GIT motility (mainly in equines), causes relaxation, hyperthermia, protrusion of the third eyelid and it has some extrapyramidal effects (e.g. convulsions, circling and chewing) (Lamont & Grimm, 2014; Burroughs et al., 2012a). It lasts for 6-8 hours and, in specific cases of immobilizations for semen collection by electro-ejaculation, ACP is not recommended because it can cause an inhibition of the ejaculation (Burroughs et al., 2012a; Plumb, 2008).

2.8.2.4.2 Azaperone

Azaperone reduces the motor activity and inhibits the CNS catecholamines, dopamine and norepinephrine (Burroughs et al., 2012a; Plumb, 2008). It causes sedation, vasodilation and bradycardia (but the pulse remains strong), has minimal effects on respiration (might improve the respiration rate) and has antiemetic properties (Plumb, 2008). However, it does not have antagonist or analgesic effects and it can cause chewing, torticollis, catalepsy and aggressive behavior in some species (e.g. the gemsbok) (Burroughs et al., 2012a; Plumb, 2008). Its vasodilation properties can block the vasoconstriction effect of the opioids, which is extremely important in species such as the elephant and the white rhino. It can be administered by IV, IM or SQ injections with short-acting effects and it lasts for 2-4 hours (with a smooth recovery) (Wolfe, 2015; Burroughs et al., 2012a). It is usually combined with opioids like etorphine in immobilization cases (Lamont & Grimm, 2014) or

used on its own as a tranquilizer to improve the welfare in transportation or the adaptation to new environments (Burroughs et al., 2012a).

2.8.2.4.3 Haloperidol

Haloperidol is a butyrophenone derivative that blocks the action of catecholamines like dopamine, causing sedation and it is mainly used on its own for transportation of antelopes as a medium-acting tranquilizer with an onset action between 5-10 minutes up to 8-12 hours (Wolfe, 2015; Burroughs et al., 2012a; Hofmeyr, 1981). Haloperidol does not cause ataxia or sleepiness but, in some antelopes (e.g. kudu, blesbok - *Damaliscus pygargus phillipsi,* or red hartebeest - *Alcelaphus buselaphus caama*) it can promote aggressive behavior. It cannot be mixed with opioids, hence why the veterinarian must not use it in immobilization cocktails (Burroughs et al., 2012a; Hofmeyr, 1981).

2.8.2.4.4 Long-Acting Tranquilizers

This particular group of tranquilizers (phenothiazine derivatives) is known as LANs – long-acting neuroleptics – and it is extremely useful in wildlife practice because of its effects: the drugs reduce anxiety, excitement and motor activity (Burroughs et al., 2012a; Fick, Mitchell & Fuller, 2007; Read, Caulkett & McCallister, 2000). Knowing that wildlife immobilizations are performed on a daily basis in SA, the use of LANs by the practitioner decreased the mortality rates in certain species (Burroughs et al., 2012a). Because of their oil compounds, LANs must not be administered as an IV injection and IM/SQ injections promote a slow absorption by the muscle tissue, reducing stress levels, trauma injuries and improving translocations and adaptation to new environments for several days with a single administration. It can also be used to control animals with aggressive behavior – particularly males in certain species – or to calm animals that are hospitalized or in rehabilitation (Burroughs et al., 2012a; Fick et al., 2007; Read et al., 2000; Diverio, Goddard & Gordon, 1996). The main problem of LANs is overdosing, which can induce side effects (e.g. chewing, torticollis, anorexia, shivering, tremors, star-gazing and hyper/hypothermia). The practitioner must administer the drugs by IM injection, preferably, using a pole syringe or a projectile dart with a lower gauge needle on (Wolfe, 2015; Burroughs et al., 2012a; Kock et al., 2012a; Fick et al, 2007). In felids, contrary to the ungulates, LANs must be carefully used for behavioral changes, mainly in cheetahs (Ramsay, 2014). This group of drugs does not have any specific antidote available but some of the side effects might be reversed with biperiden or diazepam (Burroughs et al., 2012a).

2.8.2.4.4.1 Zuclopenthixol acetate

By promoting a decrease in stress levels, zuclopenthixol is administered to improve adaptation to new environments (or confined environments for rehabilitation, e.g.)

and it can also influence the behavior of the individuals, particularly aggressive behavior (Wolfe, 2015; Read et al., 2000). It is a medium-acting tranquilizer in herbivore species such as the rhino and it must be carefully used in carnivores (Miller & Buss, 2015; Ramsay, 2014; Burroughs et al., 2012a). The effects begin 1-2 hours after administration and it can last for 3-4 days (peak of action at 36 hours after administration) with possible side effects including convulsions (Wolfe, 2015; Burroughs et al., 2012a).

2.8.2.4.4.2 Perphenazine enanthate

Due to its ester in sesame oil vehicle, the deposit of perphenazine in the muscular tissue is slowly released after 12-18 hours after administration (peak of action at 36 hours) and it can last for 7-10 days (Wolfe, 2015; Burroughs et al., 2012a). Like zuclopenthixol, perphenazine is used to reduce anxiety and stress in ungulates and to control aggressive behavior (Wolfe, 2015; Burroughs et al., 2012a; Read et al., 2000). The animals do not get drowsy and can eat/drink normally.

Sometimes, the veterinarian opts to use more than one tranquilizer to cover the entire adaptation process of the animal, for example, by combining haloperidol and perphenazine to facilitate the translocation of an ungulate and its adaptation to a new environment (Burroughs et al., 2012a).

2.8.2.5 SEDATIVES

Sedatives are commonly used in veterinary practice. Their effect depresses the CNS (not the PNS) causing drowsiness and they decrease the locomotor activity. These effects are dose dependent and the side effects are minimal (Lamont & Grimm, 2014; Burroughs et al., 2012a). These drugs are less selective than tranquilizers, promote sleep and are reversible (Wolfe, 2015; Burroughs et al., 2012a). The two main classes of sedatives are the benzodiazepines (e.g. diazepam, midazolam or zolazepam) and the α-2-adrenergic-agonists (e.g. xylazine, medetomidine and detomidine) (Lamont & Grimm, 2014; Burroughs et al, 2012a).

2.8.2.5.1 BENZODIAZEPINES

The benzodiazepines group is one of the primary sedatives used in veterinary medicine, including wildlife practice. As mentioned before, zolazepam is combined with tiletamine but other benzodiazepines such as diazepam or midazolam are also available and commonly used in wildlife immobilizations (Burroughs et al., 2012a). They promote the action of GABA, which reduces the release or the turnover of acetylcholine in the CNS. It induces depression of the CNS and promotes sedation, skeletal muscle relaxation, anxiolytic and anticonvulsive effects (Plumb, 2008). These drugs have amnesic properties, which are very useful when the veterinarian has to re-immobilize the animal in a short period of time.

However, although the properties of sedatives result in amnesic effects in human beings, in felids there is still some reluctance about these effects of benzodiazepines (Ramsay, 2014).

2.8.2.5.1.1 Diazepam

Diazepam was the first sedative to be used in the very first wildlife immobilization procedures (Burroughs et al., 2012a). It is a muscle relaxant, anxiolytic, anticonvulsant and hypnotic drug, also used as an appetite-stimulant (mainly IV) (Burroughs et al., 2012a; Plumb, 2008). In some species it can cause ataxia, increase the CNS excitement and be responsible for behavioral changes (Plumb, 2008). When mixed with other drugs, diazepam can precipitate and so it cannot be included in the cocktail dart (Burroughs et al., 2012a; Plumb, 2008). Oral diazepam, commonly used in felids, can be given as premedication with bait, 1-3 hours before the immobilization (Ramsay, 2014).

2.8.2.5.1.2 Midazolam

Midazolam is a relatively recent drug when compared with diazepam and it is more potent and effective (Burroughs et al., 2012a). It has replaced diazepam because it has a more predictable IM absorption and it can be used in wildlife practice as a top-up drug, a short-acting sedative in rhino re-location, or even in bait (Miller & Buss, 2015; Ramsay, 2014; Burroughs et al., 2012a).

2.8.2.5.2 BENZODIAZEPINE ANTAGONIST: Flumazenil

Benzodiazepines antagonists compete with benzodiazepines at the receptors level and reverse its effect in the CNS by antagonizing the sedation either in cases of therapeutic use or in overdose cases (Plumb, 2008; Walzer & Huber, 2002). Flumazenil is the most used antidote to reverse the zolazepam and promote a smooth recovery in 1-2 minutes after an IV administration (Ramsay, 2014; Burroughs et al., 2012a, Plumb, 2008).

2.8.2.6 α – 2 - ADRENERGIC AGONISTS

Alpha-2-agonists and opioids usually have a synergistic and addictive effect. At the presynaptic level of the noradrenergic neurons, α-2-agonists inhibit the norepinephrine release by bounding with the α-2-adrenoreceptors. Activity in the Sympathetic Nervous System (SNS) is reduced and it results in a decreased heart rate and blood pressure (Lamont & Grimm, 2014). It induces muscle relaxation, sedation and analgesia, and reduces the stress response. In higher doses, it can induce vomiting because of the activation of the chemoreceptor trigger zone, hypothermia (most of the time, but temperature rises can be observed in animals in warm environments), miosis and hypoxemia. By inhibition of the antidiuretic hormone, the animal usually has an increase of the urine production and a

decrease of gastrointestinal motility might result in bloat and colic problems, mainly in herbivores (Wolfe, 2015; Lamont & Grimm, 2014).

2.8.2.6.1 Xylazine

Xylazine was probably the first α-2-agonist to be used in veterinary practice. It can be administered in many species and is easily available at a low price (Lamont & Grimm, 2014; Burroughs et al., 2012a). It promotes good muscle relaxation, sedation and a short period of analgesia (Burroughs et al., 2012a; Plumb, 2008). However, it can cause hyper salivation, muscle tremors in some species, GIT motility suppression (leading to ruminal atony and bloat), vomiting in big cats, hypertension followed by hypotension and bradycardia, decreased heart contraction, respiratory depression, hyper or hypothermia according to the environment temperature, and even abortion in the last trimester. It can be reversible with yohimbine or atipamezole (Lamont & Grimm, 2014; Burroughs et al., 2012a; Plumb, 2008). The depression on the CNS is caused by a decreasing of the release of the norepinephrine and dopamine, and by the inhibition of norepinephrine release in the adrenoceptors at a presynaptic level and at a postsynaptic level in the receptors located on the peripheral vascular smooth muscle (Lamont & Grimm, 2014; Plumb, 2008). Xylazine can be combined with opioids or cyclohexylamines to improve the immobilization by reducing the doses and the induction time, and by promoting a better muscle relaxation (Lamont & Grimm, 2014; Burroughs et al., 2012a).

2.8.2.6.2 Medetomidine

Medetomidine is about 10x more powerful than Xylazine (Burroughs et al., 2012a). It is a sedative with analgesic properties commonly used in veterinary practice, preferably in carnivores (Ramsay, 2014). Because of its selective bounding with the α-2-receptors, the antidote available, atipamezole, is specifically used to reverse medetomidine (Lamont & Grimm, 2014). It can cause bradycardia, bradypnea, hypothermia, vomiting and hypotension (Lamont & Grimm, 2014; Burroughs et al., 2012a; Plumb, 2008). Medetomidine is usually combined with opioids, ketamine or TZ and administered intramuscularly, although it can be painful (Lamont & Grimm, 2014; Ramsay, 2014; Plumb, 2008).

2.8.2.6.3 Detomidine

Detomidine is a sedative also used because of its analgesic properties (Burroughs et al., 2012a; Plumb, 2008). As a long-acting sedative, it is relatively safe to be used in pregnant females and, like medetomidine, it is 10x more potent than xylazine (Lamont & Grimm, 2014; Burroughs et al., 2012a). It is less used than medetomidine and it is usually mixed with other drugs for the immobilization of specific species (e.g. zebra and white rhino) (Miller & Buss, 2015; Lamont & Grimm, 2014; Burroughs et al., 2012a).

2.8.2.7 α – 2 - ADRENERGIC ANTAGONISTS

Probably the biggest advantage of using α-2-adrenergic-agonists lies in the fact that these drugs have antidotes which can easily reverse its effects (Lamont & Grimm, 2014; Ramsay, 2014; Plumb, 2008). The veterinarian has to be aware that the analgesic effect of the α-2-agonists previously administered is also reversed with the antidote and the animal might need other extra analgesic drug after the administration of the reversal. Vasodilation and tachycardia as result of the use of reversal drugs need to be monitored (Lamont & Grimm, 2014; Plumb, 2008). In some species, the practitioner may not administer the antagonist to promote a relaxed recovery avoiding self-induced trauma lesions (Lamont & Grimm, 2014).

2.8.2.7.1 Yohimbine

Yohimbine is administered intravenously, slowly, to reverse the effects of xylazine, but it has no effect against medetomidine (Lamont & Grimm, 2014; Burroughs et al., 2012a; Plumb, 2008). It can cause muscle tremors, hyper-salivation, tachypnea and excitement of the CNS (Plumb, 2008).

2.8.2.7.2 Atipamezole

It is the first choice antidote to reverse medetomidine, due to its selectivity to α-2-adrenergic receptors (Ramsay, 2014; Plumb, 2008). It is administered as an IM (or IV) injection – the recommendations in carnivores are 2.5-5x the mg equivalent of α-2-agonits - and might cause some excitement in some cases and in ruminants, 5x the mg equivalent of α-2-adrenergic-agonits (Lamont & Grimm, 2014; Ramsay, 2014; Burroughs et al., 2012a). However, it is an expensive antidote and, occasionally, it might induce vomiting, diarrhea, hyper-salivation, tremors and aggressive behavior. In painful procedures, the practitioner must considerer the administration of additional analgesia (e.g. butorphanol) to improve the recovery because atipamezole can reverse the main analgesic properties of the α-2-adrenergic-agonists (Plumb, 2008; Lamont & Grimm, 2014).

2.8.2.8 OTHER DRUGS USED IN WILDLIFE PRACTICE

2.8.2.8.1 Doxapram

Doxapram is a temporary CNS stimulant and is used by practitioners to improve respiration by stimulation of the medullary respiratory center and through the reflex activation of the peripheral aortic and carotid sinus chemoreceptors (Burroughs et al., 2012a; Plumb, 2008). Although it's fast-acting, its effect is very short after IM or IV administration. The veterinarian must replace its action by administering butorphanol or naltrexone when the animals are still hypoxic (Burroughs et al., 2012a). In white rhinos, it must be used with

caution because it can cause excitement of the CNS and increase muscle tremors (Radcliffe & Morkel, 2014).

2.8.2.8.2 Hyaluronidase

Hyaluronidase is an enzyme that liquefies hyaluronic acid, decreases the viscosity of the connective tissue and promotes a better diffusion of the injectable drugs (Burroughs et al., 2012a; Cattet & Obbard, 2010). In wildlife practice it has been used to improve the efficacy and safety of the chemical immobilization of free-ranging animals. Hyaluronidase is added as a powder to the drugs in the dart to promote the drug(s) absorption by the muscle and reduce the induction time in several species (e.g. giraffe) (Cattet & Obbard, 2010).

2.9 COMPLICATIONS IN WILDLIFE IMMOBILIZATIONS

2.9.1 STRESS

Stressful procedures such as a chemical immobilization, physical restraint or fear of humans can lead to an acute stress response that will cause distress and influence the homeostasis of the wild animal (Arnemo et al., 2014). The two most important responses to stress are mediated by the hypothalamic-pituitary-adrenal (HPA) axis (long-term response) and by the SNS, which stimulates the adrenal glands to release catecholamines (adrenaline) to improve and prepare the body's response for the threatening situation (short-term response) (Hernandez, 2014; Meltzer & Kock, 2012; Sheriff, Dantzer, Delehanty, Palme & Boonstra, 2011; Macbeth, Cattet, Stenhouse, Gibeau & Janz, 2010; Terio, Marker & Munson, 2004). Endogenous or exogenous stressful events stimulate the HPA, which leads to a disturbance of the animal's homeostasis. As a response, the pituitary gland releases ACTH, which stimulates the *zona fasciculata* cells of the adrenal gland, resulting in an increase of glucocorticoids levels. The high levels of cortisol, the main glucocorticoid, have a long-term catabolic effect, reducing fertility, leading to stunted growth and suppression of the immune system (Hernandez, 2014; Macbeth et al., 2010; Terio et al., 2004). An increase of glucocorticoids causes a short-term 'fight or flight' stress response (adaptive) with the production of adrenaline by the adrenal medulla, which is a part of the ANS. Adrenaline increases heart rate, pulse and blood pressure, and decreases digestion and other parasympathetic nervous system responses, which will only cease when the threat is over (Meltzer & Kock, 2012; Sheriff et al., 2011; Macbeth et al., 2010). These stress-related changes can lead to major complications such as physical trauma, hyperthermia, exhaustion (capture myopathy), acidosis, shock and, ultimately, death (Arnemo et al., 2014; Hofmeyr et al., 2012).

Stress can be classified in three different phases: alarm, resistance or adaptation and exhaustion. The first stage will induce a 'fight or flight' response; at the second stage, the

stress factor is still increasing the levels of glucocorticoids, catabolic effects are present and the immune system is suppressed. Finally, at the exhaustion stage, catabolism and immune system suppression make the animal extremely vulnerable to succumb to other diseases, increasing the chances of gastric ulcers and cellular degeneration scenarios (Hofmeyr et al., 2012; Meltzer & Kock, 2012).

Maladaptation is another complication, particularly in recently re-located animals. It can be associated with stress and usually means the unsuitability of an animal to a new environment which is sometimes related with inappropriate nutrition. For example, springboks are often moved to bushveld wet areas where they succumb to problems such as heartwater because of the stress of the transport to a new habitat with a new climate (Meltzer & Kock, 2012).

2.9.2 CAPTURE MYOPATHY

Capture Myopathy (CM) is probably the most common complication in free-ranging wildlife (Wolfe, 2015; Arnemo et al., 2014; Spraker, 1993). It is a noninfectious, metabolic disease found in domesticated and non-domesticated animals which can result in death (Paterson, 2014) It has been termed in literature as: Capture Stress, Stress Myopathy, Disease Muscle Dystrophy, Exertional Myopathy, Exertional Rhabdomyolysis, Overstraining Disease, Degenerative Polymyopathy, Leg Paralysis, White Muscle Disease, Idiopathic Muscle Necrosis, Muscle Necrosis, Diffuse Muscular Degeneration or even March Myoglobinuria/Exertional Rhabdomyolysis syndrome in human beings, especially in untrained athletes following hard exercise (Blumstein et al., 2015; Paterson, 2014; Sanchez, 2011; Cattet, Stenhouse & Bollinger, 2008b; Hartup, Kollias, Jacobsen, Valentine & Kimber, 1999; Spraker, 1993). This life-threatening syndrome was described decades ago in herbivores but it is now reported in several different mammals, reptiles, fish, amphibians and avian species associated with extended pursuits, restraint techniques, re-location and other stress-inducing factors (Blumstein et al., 2015; Wolfe, 2015, Hernandez, 2014; Napier & Armstrong, 2014; Paterson, 2014; Kohn, 2013; Sanchez, 2011; Cattet, Boulanger, Stenhouse, Powell & Reynolds-Hogland, 2008a; Cattet et al., 2008b; Hartup et al., 1999; Spraker, 1993). For example the stress of being chased by a helicopter, stimulates the animal to run faster and longer, resulting in an intensive muscular activity and exhaustion which causes CM (Arnemo et al., 2014; Arnemo et al., 2006). An alteration of the blood flow to the tissues caused by a stress-inducing factor will exhaust the skeletal and cardiac muscular adenosine triphosphate (ATP – aerobic energy), reducing the delivery of oxygen to the different tissues in the body and increasing the production of lactic acid (responsible for the metabolic acidosis). This phenomenon will cause muscle necrosis by an incorrect removal of cellular waste and, consequently, myoglobin in circulation increases, leading to myoglobinuria and inappropriate renal function (Wolfe, 2015; Paterson, 2014; Meltzer &

Kock, 2012; Spraker, 1993). Other signs of CM often identified in wildlife practice include prostration, ataxia, inability to stand, hyperthermia, tachypnea, tachycardia, muscle stiffness, tremors and torticollis, paralysis, muscle pain and acute renal failure (Blumstein et al., 2015; Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Kohn, 2013; Hofmeyr et al., 2012; Sanchez, 2011). Typically, animals become unresponsive, anorectic and death can occur within minutes to hours, days, or even weeks after the immobilization event (Blumstein et al., 2015; Paterson, 2014). The word rhabdomyolysis describes the pathophysiology behind the CM syndrome. It means 'dissolution of skeletal muscle', which results in a rupture of the skeletal muscle fibers with a leakage of intracellular creatinine phosphokinase (CK) and myoglobin into the blood (Paterson, 2014).

CM can be prevented by decreasing stress levels during the immobilization, keeping handling, noise, restraint, external stimulation and danger perception to a minimum especially if predisposing factors are present (SECONDS) (Arnemo et al., 2014; Hernandez, 2014; Pas, 2014; Sanchez, 2011).

To avoid CM, the veterinarian can limit the pursuit to 3 minutes (ideally), wait for a temperature below 20ºC, reduce visual and auditory stimuli and decrease the induction and immobilization time, minimizing the excitement and avoiding underdoses (Wolf, 2015; Sanchez, 2011). However, when CM is detected, any treatment that the practitioner might administer to avoid a fatal myopathy is often insufficient to reverse the situation, hence being associated with a low success rate (Wolfe, 2015; Paterson, 2014). The animal has to be cooled down and the veterinarian must start an aggressive fluid therapy, oxygen therapy, metabolic acidosis correction with sodium bicarbonate, analgesics and muscle relaxants (benzodiazepines such as midazolam or central-acting muscle relaxants like methocarbamol), and vitamins (vitamin E and selenium, mainly but others might also be administer). Some associations of corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) can be administered to protect vascular integrity (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Hofmeyr et al., 2012; Sanchez, 2011). A number of authors recommend the administration of dantrolene sodium, a lipid soluble hydantoin analog that prevents malignant hyperthermia in humans and the exertional rhabdomyolysis in horses by suppressing the calcium release from the sarcoplasmic reticulum. However, there are no reports of its effects in wildlife yet (Paterson, 2014).

CM can be classified in four different categories: (Blumstein et al., 2015; Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Sanchez, 2011; Spraker, 1993)

2.9.2.1 Hyperacute or Capture Shock Syndrome

Shock is the term used by the veterinary practitioners to describe a terminal stage of organs' failure, such as a drop in blood pressure as a result of blood volume loss, severe dehydration or high levels of parasitism (Meltzer & Kock, 2012). During the

immobilization, an acute death syndrome known as capture shock syndrome might occur within 1-6 hours postcapture. The animal shows signs of tachypnea, tachycardia, hyperthermia, hypotension, depression and ultimately death (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Sanchez, 2011). By analyzing the serum enzymes the practitioner will detect an increase of the aspartate aminotransferase (AST), of the CK and an increase of the lactate dehydrogenase (LDH) (Paterson, 2014). Postmortem findings might include hepatic congestion, pulmonary congestion and edema, small intestine congestion with blood-tinged contents in the lumen. Histologically, areas of necrosis will occur in the skeletal muscle, brain, liver, heart, adrenal glands, spleen, pancreas, kidneys and lymph nodes (Wolfe, 2015; Hernandez, 2014; Paterson, 2014).

2.9.2.2 Ataxic Myoglobinuric Syndrome

Is the most commonly observed of the four syndromes and it can occur hours or days after the immobilization (Paterson, 2014). Severe clinical signs of ataxia, torticollis and myoglobinuria might be visible (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Sanchez, 2011; Spraker, 1993). Serum enzymes AST, CK, LDH and BUN (blood urea nitrogen) levels are abnormally increased. The kidneys are dark and swollen, with dark content, tubular necrosis and myoglobin casts; and the skeletal muscle has multifocal, pale, soft, dry areas, associated with hypoxia and ATP deficiency, which results from an exhaustion of the aerobic glycolysis (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Spraker, 1993). Animals with moderate to severe signs have a higher chance of dying (Paterson, 2014).

2.9.2.3 Ruptured Muscle Syndrome

Occurs within 24-48 hours postcapture. Clinical signs might include a drop of the hindquarters and uni or bilateral hyperflexion of the hock, caused by the rupture of gastrocnemius muscle, middle and deep gluteal, semitendinosus or semimembranosus muscles (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Sanchez, 2011; Spraker, 1993). In the serum, AST, CK and LDH are extremely increased but BUN is often normal or slightly increased. The animal shows signs of SQ hemorrhage of the rear limbs, muscle necrosis and multifocal pale lesions on the forelimb, hind limb, diaphragm, cervical and lumbar muscles (Paterson, 2014). The type of lesions found in animals with this syndrome is also compatible with vitamin E and selenium deficiency, which can be related to CM. Individuals with this form of CM might resist and survive for some weeks but most of them will die (Hernandez, 2014; Spraker, 1993).

2.9.2.4 Delayed-Peracute Syndrome

This is a rare syndrome that could appear more than 24 hours after the immobilization procedure (Paterson, 2014). Although the animals seem to be normal, sudden

death occurs due to a ventricular fibrillation and high levels of AST, CK and LDH (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Sanchez, 2011; Spraker, 1993). There are no visible lesions but sometimes small pale foci on the skeletal muscle are detected, characterized by a mild to moderate rhabdomyolysis throughout the skeletal muscle, particularly in the hind limbs (Wolfe, 2015; Hernandez, 2014; Paterson, 2014; Spraker, 1993).

Differential diagnosis for CM in wildlife might include plant toxicity, malignant hyperthermia, early tetanus, hypocalcemia or myositis (Paterson, 2014).

2.9.3 RENARCOTIZATION

Renarcotization is a particular complication in wildlife medicine, particularly in herbivores immobilized with opioids (Napier & Armstrong, 2014; Pas, 2014). Animals that have already been immobilized and reversed with an antidote will show signs of re-sedation hours after the reversal has been administered (Pas, 2014). Antagonists like diprenorphine to reverse carfentanil or thiafentanil might cause re-narcotization. Because of its long half-life, naltrexone is a much safer choice if the practitioner intends to re-immobilize the animal without the chance of the animal being re-sedated after the antidote has been administered (Napier & Armstrong, 2014; Pas, 2014). But the veterinarian must use the correct doses of antidote to reverse the opioid otherwise an underdose might cause renarcotization (Napier & Armstrong, 2014).

Opioid levels in the blood will maintain the animal's clinical signs for 12-24 hours after the administration of the reversal. Some of the signs might include excitement, circling, high stepping, recumbency paddling and, consequently, hyperthermia, acidosis and exhaustion. In these situations, the veterinarian needs to repeat the antidote by IM administration of half of the dose. Sometimes, if the second dose of antidote does not reverse the animal completely, the practitioner must repeat the dose until the clinical signs of renarcotization cease (Napier & Armstrong, 2014).

2.9.4 PHYSICAL TRAUMA

An immobilization procedure, with physical or chemical restraint, can be traumatic for the animal involved. Injuries such as lacerations, fractures or abrasions can be accidentally inflicted on the animal, by itself, by other animals or by the team in charge. A proper handling and a correct prediction of the animal’s behavior as well as previous knowledge of the environmental hazards might prevent several physical traumas (Arnemo et al., 2014; Arnemo et al., 2006). Trauma can even lead to death in capture procedures and a loss of 10 % of the animals in an immobilization is expected. However, when immobilization involves endangered species, a loss of a single individual can be dramatic and this percentage must not be accepted (Hernandez, 2014; Meltzer & Kock, 2012).

2.9.5 HIPOXEMIA AND HYPOXIA

One of the most common complications that can occur during immobilization is hypoxemia (low arterial oxygen tension) brought on by hypoventilation, which can be a result of respiratory depression caused by some immobilization drugs. It may also be a consequence of loss of lung volume, possibly resulting from an incorrect recumbency position. In herbivores, for example, the GIT content presses the diaphragm forward, decreasing the volume of the lungs and compromising the expansion of the thorax on inspiration (Arnemo et al., 2014; Fahlman, 2014; Lamont & Grimm, 2014; Napier & Armstrong, 2014; Pas, 2014; Cracknell, 2014; Meltzer & Kock, 2012; Bush, Raath, Grobler & Klein, 2004). An unusual cause of hypoxemia is pneumothorax, which can happen after a dart penetration into the thoracic cavity or a puncture of a horn (Arnemo et al., 2014; Arnemo et al., 2006). Hypoxia, the inadequate oxygen level in the body tissues, is a consequence of hypoxemia when the oxygen delivery is not improved (by increasing the cardiac output or by decreasing the oxygen consumption in the tissues). This status results in rapid cellular degeneration in organs like the brain, heart, kidneys and liver. A lower perfusion of the tissues with low levels of hemoglobin can also be responsible for hypoxic status because the tissues might not be able to use oxygen properly or because the hemoglobin is not able to transport the oxygen to the tissues (Fahlman, 2014; Cracknell, 2014).

2.9.6 HYPERTERMIA/HYPOTHERMIA

Overheating during an immobilization procedure in wildlife practice is an expected complication. Hyperthermia can result from high environmental temperatures, especially during summer or during the middle of the day. It occurs due to excessive muscular exercise and muscular contraction, when the animals are being chased or frightened, or from the drugs used, which interfere with the thermoregulatory center (Arnemo et al., 2014; Schumacher, 2008; Burroughs & McKenzie, 1993). The ideal temperature for immobilizations should be around 25ºC (Meltzer & Kock, 2012). When its temperature rises above 43ºC the animal might show signs of panting, weakness, arrhythmia, shallow breathing, convulsions or even die. A rectal temperature of more than 41ºC is considered an emergency and, in these cases, the practitioner has to act (Arnemo et al., 2014; Meltzer & Kock, 2012; Schumacher, 2008). To avoid this situation or to cool down the temperature, the animal has to be moved to a shaded area and sprayed with cold water (or cooled in a river, with snow, fluids or enemas), supplied with oxygen (because hyperthermia increases the oxygen demand) or keep stress to a minimum by avoiding an intense chase or stressful restraint methods (Arnemo et al., 2014; Hofmeyr et al., 2012; Schumacher, 2008). The practitioner must always evaluate the conditions and decide if the immobilization will go ahead or not (Arnemo et al., 2014; Hofmeyr et al., 2012; Meltzer & Kock, 2012).

Hypothermia is characterized in mammals by a temperature below 35ºC and while not as common as hyperthermia, can also occur, particularly if the immobilization is taking place in areas with low temperatures, wet, rainy environments or when the animals immobilized are young, small or in poor body condition (Arnemo et al., 2014; Schumacher, 2008). This condition can be reversed by warming the animal up with hot water bottles or by drying the animal. To prevent hypothermia, weather conditions such as exposure to wind or low temperatures must be avoided (Arnemo et al., 2014).

2.9.7 BLOAT

In ruminants, a correct recumbency position (sternal) during an immobilization procedure is vital, otherwise in a lateral position bloat can occur. Another cause of bloat is the use of immobilization drugs such as the α-2-agonists, which will result in a ruminal atony and, consequently, bloat. That tympanic stage happens due to the inability to expel gases through eructation. If the immobilized animal starts to bloat, it must be re-positioned into a sternal recumbency with the neck extended and the head with the nose pointing down to drain the saliva. Intubating the animal to relieve the gases inside might be an option, or even a trocharization of the rumen. If the problem is caused by α-2-adrenergic-agonists, the veterinarian might use the antidotes available to antagonize their effect (Arnemo et al., 2014; Napier & Armstrong, 2014; Hofmeyr et al., 2012; Arnemo et al., 2006).

2.9.8 VOMIT/REGURGITATION AND ASPIRATION PNEUMONIA

Vomiting and regurgitation are considered emergencies since they might entail the possibility of aspiration pneumonia. Vomiting could be a side effect of opioids or α-2-adrenergic-agonists administration. As mentioned before, the correct recumbency position is an important aspect in wildlife practice. While ruminants are usually in a sternal position with the neck straight and the nose down, carnivores are positioned laterally. If the practitioner notices that the animal has aspirated the stomach/rumen content and might develop pneumonia, he will need to administer a broad spectrum long-acting antibiotic (Arnemo et al., 2014; Pas, 2014; Hofmeyr et al., 2012; Arnemo et al., 2006).

2.9.9 EQUIPMENT FAILURE

Notwithstanding complications that can stem from an inadequate planning of the immobilization, selecting the right drug(s) and dose, or even complications associated with inexperienced personnel, equipment failure is always a factor that still can occur during a procedure (Arnemo et al., 2014; Atkinson et al., 2012). Darts form part of basic equipment for wildlife immobilizations but can still suffer a number of failures and problems during procedures (Hernandez, 2014; Shury, 2014). When the needle impacts on the animal it can bend or even break, meaning the drug(s) might be inadequately administered. An

inappropriate loading of the dart in the gun might cause an incorrect projection, while a loss of the tailpiece can cause a loss of balance. Power charges might not work properly because of the weather conditions and the carbon dioxide or the compressed-air cylinders may leak. The practitioner might miss the target or hit the animal in the wrong place (SQ tissue, fat deposit, chest or abdomen, bone or vital organs).The strength of impact/velocity of impact can influence the injection of the content into the animal. The dart gun and the dart might be rusty or dirty; the dart wound might cause infections or a misplaced dart might even cause a pneumothorax (Arnemo et al., 2014; Hernandez, 2014; Atkinson et al., 2012; Arnemo et al., 2006).

CHAPTER 3.
PRACTICAL CASES

3.1 INTRODUCTION

Given that the goal of this thesis is to review the main principles of wildlife immobilization, the anesthetic protocols used on the various African species immobilized are presented in this chapter, along with the doses and the characteristics of the darts/ dart guns involved in each capture procedure.

Graphic 1 shows the percentage of the different species examined during the internship. Most of the species were ungulates (85.32%) and only 14.68% were carnivores. The African buffalo was the most immobilized species, followed by the sable antelope, the blue duiker and the African lion. At the other end of the spectrum, species such as the leopard, the white rhino or the bushbuck were only immobilized once. Table 2 represents the

exact number of individuals restrained and the respective number of chemical immobilization done for each species approached.

Graphic 1 – Percentage of individuals from different species examined during the internship for veterinary procedures.

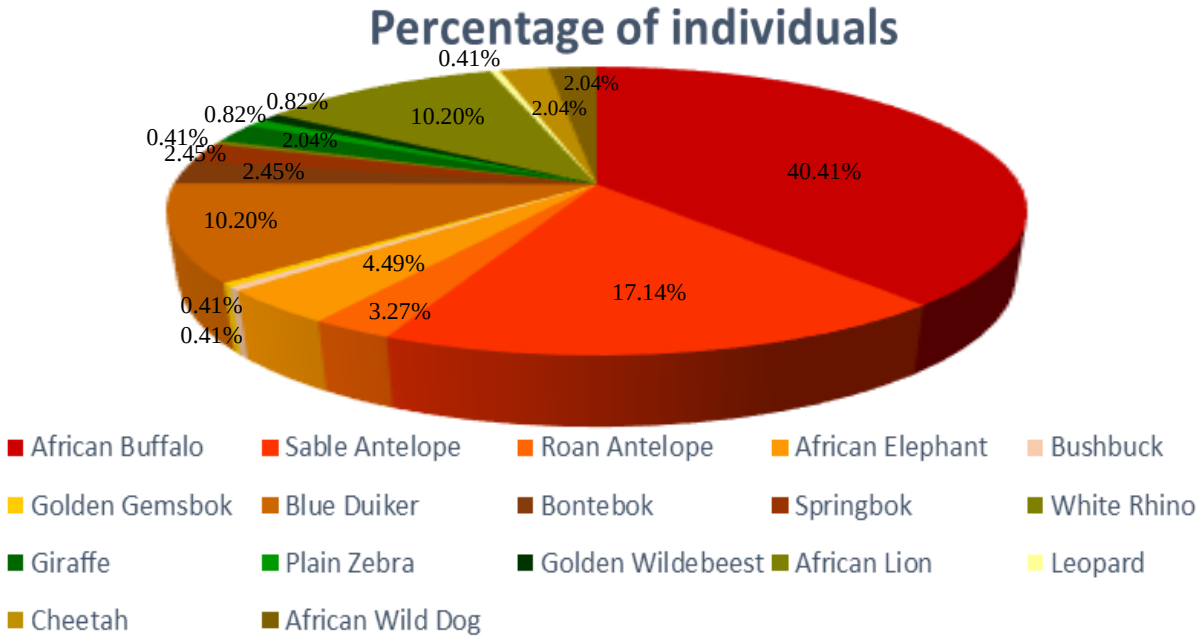


Table 2 – Number of animals, divided by species, immobilized during the internship (total of 245 animals and 184 chemical immobilizations).

SPECIES	African Buffalo	Sable Antelope	Roan Antelope	African Elephant	Bushbuck	Golden Gemsbok	Blue Duiker	Bontebok	Springbok	White Rhino	Giraffe	Plains Zebra	Golden Wildebeest	African Lion	Leopard	Cheetah	African Wild Dog
Number of individuals	99	42	8	11	1	1	25	6	6	1	5	2	2	25	1	5	5
Number of chemical immobilizations	98	31	8	3	1	0	0	6	5	1	5	2	2	14	1	2	5

This large diversity of species is representative of the various procedures performed. All the procedures were grouped in fourteen categories according to the species immobilized (Table 3).

Table 3 – Different activities performed during the internship divided according to the species and the procedure.

SPECIE	Total of individuals	Transport, re-location, off-load or boma	Antibiotics, vitamins, de-worming	Microchip	Measurements	Ear Tag	Implants	GPS Collar	Surgery	Vaccination	Tests: TB, GnRH, DNA, coprology	X-rays, US, Pregnancy exams	Necropsies, Eutanasia	Certifications	Wound treatments, check ups	TOTAL
African Buffalo	99	16	99	22	61	22	0	0	0	95	21	54	1	0	1	392
Sable Antelope	42	28	35	19	13	3	0	0	0	25	16	5	0	0	2	146
Roan Antelope	8	2	8	4	5	2	0	0	0	4	4	0	0	0	0	29
African Elephant	11	0	1	0	0	0	0	0	2	6	4	0	0	1	1	15
Bushbuck	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	3
Golden Gemsbok	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Blue Duiker	25	0	25	25	0	25	0	0	0	0	25	0	0	0	1	101
Bontebok	6	0	6	6	0	6	0	0	0	0	6	0	0	0	0	24
Springbok	6	5	5	0	0	0	0	0	1	0	0	0	0	0	0	11
White Rhino	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2
Giraffe	5	5	4	0	0	0	0	0	0	0	0	0	1	0	0	10
Plains Zebra	2	0	2	0	0	0	0	0	3	0	0	0	0	0	0	5
Golden Wildebeest	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	6
African Lion	25	3	16	0	0	0	5	3	5	13	0	0	0	0	3	48
Leopard	1	0	1	0	0	0	0	0	2	0	0	0	0	0	0	3
Cheetah	5	1	2	1	1	0	0	0	0	4	1	0	0	0	0	9
African wild Dog	5	5	5	4	1	0	0	0	0	4	0	4	0	0	0	23
TOTAL	245	69	212	82	82	58	5	3	14	151	77	63	3	1	9	829

GPS (Global Positioning System), TB (Bovine Tuberculosis), GnRH (Gonadotropin-Releasing Hormone), US (Ultra-Sound)

3.2 METHODS, MATERIALS AND RESULTS

The tables presented later on explain the different anesthetic protocols used on the various African species immobilized during the internship. The doses of the drugs (used for immobilization, used as reversals and as additional administrations) and the characteristics of the darts/ dart guns involved in each procedure are tabled along with the results (immobilization succeeded or not succeeded) for each protocol performed. Additional comments are also provided about the results obtained.

The weight of the species was established according to the references from Miller & Fowler (2015) except for the male bushbuck. Its weight is based on a reference by Kock & Burroughs, 2012. As can be seen below, for ungulates, the doses are tabled as net doses in mg and not associated with the weight of the animal (mg/kg) because the various protocols published by the main wildlife practitioners are also in net doses references (see Appendix 2. Table 20-34, where the different protocols suggested by the main wildlife practitioners are tabled).

3.2.1 AFRICAN UNGULATES

3.2.1.1 AFRICAN BUFFALO

The African buffalo is one of the most commonly immobilized species in SA because of its impact on livestock (and vice-versa). In terms of diseases legally requiring testing, bovine tuberculosis (TB), brucellosis, foot and mouth disease (FMD) and corridor disease (Theileriosis by *Theileria parva*) are the most important ones (Tanner et al., 2015; Anderson, Ezenwa & Jolles, 2013; Beechler, Broughton, Bell, Ezenwa & Jolles, 2012; Burroughs et al., 2012b; Pienaar, Potgieter, Latif, Thekisoe & Mans, 2011; Chaisi, Sibeko, Collins, Potgieter & Oosthuizen, 2011; Ayebazibwe et al., 2010; Sibeko et al., 2008; De Klerk et al., 2006; Oosthuizen, 2006; Jolles, Cooper & Levin, 2005; Kalema-Zikusoka, Bengis, Michel & Woodford, 2005; Anderson, Foggin et al., 1993; Anderson, Doughty, Anderson & Paling, 1979; Hedge, 1972).

Figure 10 – Herd of buffalos in a Game Farm (semi-controlled environment) (Original).

During the internship, most of the buffalos examined were in large parks/semi-controlled environments, sometimes in bomas (Figure 10) and any time an animal did not go down after the first dart, a second dart with the same dose or with half of the dose was given (Table 4).

Table 4 – African buffalo's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Bull (300-900kg)	22	*Pneu Dart* gun type C	2cc darts 1.5'' WB	Thiafentanil:4mg + Etorphine:4mg (+ Medetomidine:1-2mg) + Azaperone: 40-60mg	Diprenorphine:3 x Etorphine + Naltrexone: 10x Thiafentanil Or Naltrexone: 10x Thiafentanil	Well succeeded	IV administration of 7.5mg of Midazolam if needed; IV administration of Medetomidine 1mg if needed (and reversed with Atipamezole 5mg); 1 individual required a 2nd dart
Cow (300-900kg)	55			Thiafentanil:3mg + Etorphine:3mg (+ Medetomidine:1mg) + Azaperone: 40-60mg		Well succeeded	IV administration of 7.5mg of Midazolam if needed; 2 individuals required a 2nd dart
Juvenile	5			Thiafentanil: 2.5-3.5mg + Azaperone:40mg		Well succeeded	-
Calf	16		1cc darts 3/4'' WB	Thiafentanil: 1.5mg		Well succeeded	-

Note:
Drugs with ( ) were not administered to all animals
Thiafentanil (*Thianil*®, 10mg/m, Wildlife Pharmaceuticals, Mpumalanga, SA)
Etorphine (*M99*®, 9.8mg/mL, Novartis, Vorna Valley, SA)
Naltrexone (*Trexonil*®, 50mg/mL, Wildlife Pharmaceuticals, Mpumalanga, SA; *Naltrexone*®, 50mg/mL, Kyron Labs, Johannesburg, SA)
Diprenorphine (*M5050*®, 12mg/mL, Novartis, Vorna Valley, SA)
Medetomidine (*Medetomidine*®, 20mg/mL, V-Tech, Pretoria, SA; or Domitor®, 1mg/mL, Pfizer, Port Elizabeth, SA)
Atipamezole (*Antisedan*®, 5mg/mL, Pfizer, Port Elizabeth, SA)
Azaperone (*Stresnil*®, 40mg/mL, Bayer, Isando, SA)
Midazolam (*Midazolam*®, 15mg/3mL, Roche Products, Illovo, SA)

3.2.1.2 SABLE ANTELOPE

Most of the sables were chemically immobilized in large parks but the smallest ones were inside bomas. Contrary to others captures, sable and roan antelopes were not blindfolded but, because of their horns, they required horn protections (plastic pipes) to improve the safety of the procedure for the personnel. Zuclopenthixol (*Clopixol-Acuphase*®, 50mg/mL, Lundbeck Limited, North Riding, SA) or haloperidol (*Haloperidol*®, 20mg/mL, V-Tech, Pretoria, SA) IM were also administered for re-location purposes, allowing an improvement during the transportation and a better adaptation to a new environment (Table 5).

Table 5 – Sable antelope's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Bull (190-300Kg)	19	*Pneu Dart* gun type P	2cc darts 3/4'' WB	Thiafentanil:6-7mg + Azaperone:40-60mg	Naltrexone 10x Thiafentanil	Well succeeded	IV administration of 5-15mg of Midazolam if needed; IV administration of Medetomidine 1mg if needed (and reversed with Atipamezole 5mg)
Cow (190-300Kg)	6			Thiafentanil:6mg + Azaperone:40-60mg		Well succeeded	IV administration of 5-15mg of Midazolam if needed; IV administration of Medetomidine 1mg if needed (and reversed with Atipamezole 5mg)
Juvenile	4			Thiafentanil: 3.5mg + Azaperone:40-60mg		Well succeeded	-
Calf	2		1cc darts 3/4'' WB	Thiafentanil:1.5mg + Azaperone: 40-60mg		Well succeeded	-

3.2.1.3 ROAN ANTELOPE

All the roan antelopes were chemically immobilized in large parks and approached by foot or by vehicle (Table 6). The animals immobilized were adult males for DNA testing or bio-measurements.

Table 6 – Roan antelope's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Bull (190-300Kg)	8	*Pneu Dart* gun type P	2cc darts 1+1/4'' WB	Thiafentanil:7mg + (Medetomidine:1-2mg) + Azaperone: 40-60mg	Naltrexone 10x Thiafentanil + Atipamezole 5xMedetomidine	Well succeeded	IV administration of 5-15mg of Midazolam if needed; And/or 1 mg of Medetomidine (reversal:Atipamezole 5mg); 2 individuals required a 2nd dart

Note:

Drugs with ( ) were not administered to all animals

3.2.1.4 SPRINGBOK

The springboks immobilized were darted by foot and by vehicle in non-controlled environments. They were captured for re-location purposes (only one was physically restrained and it is not part of the samples in Table 7), and haloperidol was given IM to the individuals (Table 7).

Table 7 – Springbok's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Ram (30-45Kg)	1	*Pneu Dart* gun type P	1cc darts 1'' GC	Thiafentanil:1.5-1.7mg+ Azaperone:20mg	Naltrexone 10x Thiafentanil	Well succeeded	-
Ewe (30-45Kg)	4			Thiafentanil:1.5mg+ Azaperone:20mg		¼ not succeeded; ¾ well succeeded	One death: not related to the anesthetic protocol – Drowned in a lake during the excitement phase

3.2.1.5 BONTEBOK

Due to the slight visual differences between a bontebok and a blesbok (*Damaliscus pygargus phillipsi*), many owners in SA want their animals tested to make sure the individuals are not a result of an unwanted cross-breeding (van Wyk, Kotzé, Randi & Dalton, 2013; Van der Walt, Nel & Hoelzel, 2001). The free-ranging bonteboks examined were approached by vehicle and chemically immobilized for DNA testing (Table 8).

Table 8 – Bontebok's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS
Ram (55-80Kg)	3	*Pneu Dart* gun type P	1cc darts 1'' GC	Thiafentanil:3mg+ Azaperone:20-40mg	Naltrexone 10x Thiafentanil	Well succeeded
Ewe (55-80Kg)	3					Well succeeded

3.2.1.6 GIRAFFE

Giraffe capture is a complex and dangerous procedure. Usually, a free-ranging giraffe is chemically immobilized mainly for re-location purposes because of the high risk of complications during restraints that could result in death. The goal of this restraint relies on a fast and efficient darting, preferably by helicopter, with a very high dose of opioid (Table 9). Excessive running must also be avoided (Bertelsen, 2015; Citino & Bush, 2014; Citino, Bush, Lance, Hofmeyer & Grobler, 2006; Bush, 2003; Bush et al., 2001; Bush, Raath, Phillips & Lance, 1997; Bush, 1993; Morkel, 1993; Morkel, 1992; Bush & de Vos, 1987; Bush, 1976; Bush, Ensley, Mehren & Rapley, 1976). During captures, the giraffes were always approached by helicopter.

Table 9 – Giraffe's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Cow (700-1200Kg)	1	*Pneu Dart* gun type C	2cc darts 2''WB	Etorphine:10mg + Butorphanol: 10mg + Hyaluronidase: 1500-3000 IU	Naltrexone 10x opioid	Well Succeeded	-
Juvenile	4		2cc 1.5''WB	Etorphine:4-6mg + Butorphanol: 5mg/ Etorphine:4-5mg + Thiafentanil:2mg + Butorphanol:15mg + Hyaluronidase: 1500-3000 IU		¾ Well succeeded; ¼ Not succeeded	One death: not related with the anesthetic protocol – Fall into a mud pit during the excitement phase

Note:
Butorphanol (Butorphanol® 50mg/mL, Kyron Labs, Johannesburg, SA or Butorphanol® 10mg/mL, Kyron Labs, Johannesburg, SA)
Hyaluronidase (Hyalase®, 5000 IU, Kyron Labs, Johannesburg, SA)

3.2.1.7 AFRICAN ELEPHANT

Although there is no published protocol for a standing sedation on a free-ranging African elephant, in controlled environments the standing sedation is a common procedure (Wiedner, 2015; Horne, Loomis, 2014; Fowler & Mikota, 2006; Neiffer et al., 2005; Du Toit, 2001; Ramsay, 2000; Raath, 1993). The standing sedations were carried out in a semi-controlled environment, to perform surgery on the tusks and blood collection for testosterone levels testing (Table 10).

The free-ranging bull elephant was darted with an experimental cocktail for standing sedations (Figure 11).

Figure 11 – Free-ranging bull elephant sedated for blood collection and GnRH administration (Original).

Table 10 – African elephant's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	RESULTS	COMMENTS	REVERSAL
Bull (4000-7000Kg)	2 (controlled environment)	*Dan Inject* pump pistol	5cc darts 2''	Etorphine:3mg + Medetomidine:40mg + Butorphanol:150mg and Medetomidine:40mg +Butorphanol:150mg	One succeeded (1st protocol); one not succeeded (2nd protocol)	For the not succeeded immobilization: 2nd dart with 30mg Medetomidine + 75mg Butorphanol and top up (IV): 10mg Medetomidine + 25mg Butorphanol	Naltrexone 150mg (IV) + Atipamezole 25mg (2/3 IM + 1/3 IV)
	1 (free-ranging)	*Pneu Dart* gun type C	5cc darts 2'' WB	Etorphine:4mg + Medetomidine:50mg +Butorphanol:150mg + 5000 IU Hyaluronidase	Not succeeded	2nd dart: 14mg Etorphine 100 mg Azaperone + 5000 IU Hyaluronidase	Naltrexone 140mg

3.2.1.8 WHITE RHINOCEROS

The female white rhino darted for horn trimming was in a game reserve where the white rhinos had had anesthetic complications in the past. For this reason, additional measures were taken into account, particularly the choice of the protocol and respective doses (Table 11). The animal was darted from a vehicle and followed carefully after that. Because no evidence of sedation was noticed, a second dart was administered following the same protocol.

Table 11 – White rhino's anesthetic protocol and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	RESULTS	COMMENTS
Cow (1800-2200Kg)	1	*Pneu Dart* gun type C	2cc dart 2'' WB	Etorphine:3.5mg + Azaperone: 20mg + Hyalurinidase: 2500 IU	Not succeeded	2nd dart with same protocol (effective) but the animal died during anesthesia

3.2.1.9 GOLDEN WILDEBEEST (Blue Wildebeest)

The Golden wildebeest is a color variation of the blue wildebeest species (van Hoven, 2015). The two individuals darted were males, both immobilized for re-location purposes (Table 12). A drone followed one of them during the entire procedure to guarantee that the animal would not get lost after darting.

Table 12 – Golden wildebeest's anesthetic protocol and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Bull (110-180kg)	2	*Pneu Dart* gun type P	1.5cc darts ¾'' WB	Thiafentanil: 3.5-4mg + Azaperone: 40mg	Naltrexone 10xThiafentanil	Well succeeded	Zuclopenthixol: 100mg was given IM

3.2.1.10 PLAINS ZEBRA

Two Plains zebra (a stallion and a mare) were darted on foot and immobilized in order to perform hooves trimming and castration on the stallion (Table 13).

Table 13 –Plains zebra's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS
Stallion (175-275kg)	1	*Pneu Dart* gun type P	2cc darts ¾'' WB	Etorphine: 6mg + Azaperone: 60mg	Diprenorphine 3xEtorphine	Well succeeded
Mare (175-275 kg)	1			Etorphine: 5.5mg + Azaperone: 60mg		Well succeeded

3.2.1.11 BUSHBUCK

The male bushbuck was immobilized for re-location purposes after being found stuck in a private backyard (Table 14).

Table 14 – Bushbuck's anesthetic protocol and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS
Ram (40-50Kg)	1	*Pneu Dart* gun type C	1cc dart 1'' GC	Thiafentanil: 1mg + Medetomidine (Domitor®): 1mg	Naltrexone 10xThiafentanil + Atipamezole 5x Medetomidine	Well succeeded

3.2.2 AFRICAN CARNIVORES

3.2.2.1 AFRICAN LION

The African lions immobilized during the internship were restrained for different reasons and in different environments (Figure 12). The approach was done on foot or by vehicle, and most of them were darted after a carcass (bait) had been offered to improve the accuracy of darting (Table 15).

Figure 12 – Anesthetized African lion in Gondwana Game Reserve (Original).

Table 15 – African lion's anesthetic protocol and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Lion (120-250Kg)	5	*Pneu Dart* gun type C or type P	1.5cc darts 1''WB; 2cc ¾'' WB; 2cc 1+1/4'' WB	TZ: 0.4-0.5mg/kg + Medetomidine: 0.03-0.04mg/kg	Atipamezole 3x Medetomidine	Well succeeded	The animals that went to surgery (2) required top-ups of Medetomidine 0.01-0.015 mg/kg and/or TZ 0.15-0.2mg/kg (IV or IM)
Lioness (120-250Kg)	9		1.5cc darts 1''WB; 2cc ¾'' WB; 2cc 1+1/4'' WB	TZ: 0.4-0.7mg/kg + Medetomidine: 0.04-0.07mg/kg	Atipamezole 3x Medetomidine	Well succeeded	The animals that went to surgery (3) required top-ups of Medetomidine 0.01-0.015 mg/kg and/or TZ 0.15-0.2mg/kg (IV or IM) *; 2 individuals required a 2nd dart with half of the dose

Note:

TZ : Zoletil 100®, 100mg/mL, Virbac, Halfway House, SA (Tiletamine 50% + Zolazepam 50%)

* Two of the three surgeries performed on lionesses were hemi-hysterectomies in 2 members of the same pride and, for that reason they were both immobilized at the same time along with the male. While the first surgery was being performed, the second lioness was under isoflurane maintenance + TZ + Medetomidine.

3.2.2.2 CHEETAH

Cheetahs were immobilized in controlled environments: boma or enclosures (Table 16). Even on free-ranging animals this method is always good practice to improve darting opportunities. Other advice for adequate immobilization of carnivores might include a top-up to maintain the desired level of anesthesia, guarantee the safety of the personnel. Eye lubricant should be applied before the animal is blindfolded, and the practitioner must keep an eye on the animal until it gets up, recovers from anesthesia and, if possible, keep monitoring it for the next 24 hours (Lamberski, 2015; Ramsay, 2014; Crosier et al., 2007; Stegmann & Jago, 2006; Wack, 2003).

Table 16 – Cheetah's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Male (35-72Kg)	1	*Pneu Dart* gun type P	1cc darts 1''GC	TZ : 0.6mg/kg + Medetomidine:0.05mg/kg	Atipamezole 3x Medetomidine	Not succeeded	The individual required a 2nd dart + Top-up administrations with Medetomidine: 0.01mg/kg
Female (35-72Kg)	1			TZ: 0.5mg/kg + Medetomidine:0.05mg/kg	Atipamezole 3x Medetomidine	Well succeeded	-

3.2.2.3 LEOPARD

The male black leopard immobilized was in a controlled environment and surgery was performed on this individual (Figure 13) as well as nail clipping due to excessive claw growing (Table 17).

Figure 13 – Castration performed by Dr Brendan Tindall on a male black leopard (Original).

Table 17 – Male leopard's anesthetic protocol and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Male (23-91kg)	1	*Pneu Dart* gun type P	1cc darts 1''GC	TZ: 0.5mg/kg + Medetomidine: 0.05mg/kg	Atipamezole 3x Medetomidine	Not Succeeded	Top-ups required: 1st top up: TZ: 0.15mg/kg + Medetomidine:0.01mg/kg (2nd dart); 2nd top up: TZ: 0.3mg/kg + Medetomidine:0.01mg/kg (Pole syringe); 3rd top up: TZ: 0.3mg/kg + Medetomidine: 0.005mg/kg (hand-held injection)

3.2.2.4 AFRICAN WILD DOG (Painted hunting dog)

The painted dogs are usually found in packs (Scott & Kreeger, 2014; Larsen, Kreeger, West, Heard & Caulkett, 2007; Courchamp, Rasmussen & Macdonald, 2002; de Villiers, van Jaarveld, Meltzer & Richardson, 1997) which might require a complete restraint of all the individuals of the group at the same time to ensure the safety of the personnel and the animals. As an endangered species in SA, a safe chemical restraint of all the immobilized canids is critically important (Courchamp et al., 2002; de Villiers et al., 1997; van Heerden, Burroughs, Dauth & Dreyer, 1991). Four of the five immobilizations performed started with display of bait (carcass) to improve the accuracy of the darting (Table 18).

Table 18 – African wild dog's anesthetic protocols and results.

SAMPLE	NUMBER OF ANIMALS	DART GUN	DART TYPE	PROTOCOL	REVERSAL	RESULTS	COMMENTS
Male (19-35Kg)	1	*Pneu Dart* gun type P	1cc darts ¾'' WB	TZ: 1mg/Kg + Medetomidine: 0.06mg/kg	Atipamezole 3x Medetomidine	Well succeeded	-
Female (19-35Kg)	4					¼ Not succeeded; ¾ Well succeeded	Administration of a 2nd dart on the unsuccessful immobilization with 1/2 of the dose after some signs had been detected but the animal was not sedated enough to tolerate an approach + Medetomidine:0.02mg/kg

CHAPTER 4. DISCUSSION

As evident in the results from Table 2 (Chapter 3), some of the restraints performed did not require any chemical immobilization, such as the ones performed on blue duikers. A physical restraint after a net capture was the preferable method used for immobilization of these individuals. This technique is also adequate for other species, as long as an experienced team is restraining the animals to avoid injuries, excessive stress levels and other complications (Hernandez, 2014; Shury, 2014; Goodman et al., 2013; Atkinson et al., 2012; La Grange, 2012; Fivaz & Ebedes, 2012; Bothma & Van Rooyen, 2005).

However, most of the procedures carried out required a chemical immobilization. Each individual protocol and respective doses administered were mainly based on the personal experience of the veterinarian as opposed to data from academic references. The differences can be seen in the comparisons of the protocols and respective doses with the references on Appendix 2. (Table 20-34).

By analyzing the results from the chemical immobilizations performed (184), it is possible to affirm that most of the clinical cases were well succeeded, without any requirement of additional darts (171/184: 92.93%). Only a small percentage, 7.07% (13/184) of the procedures needed a second dart dose.

A higher administration of top-up anesthetics to improve the depth of the anesthesia was required in some cases. However, as was already expected, during surgery, particularly in surgical cases involving felids, the administration of drugs to maintain the anesthesia was common to ensure a safer procedure for the animals and for the team involved. Additional drugs were also provided in specific cases after a first cocktail of drugs were darted (e.g. administration of midazolam in some of the African buffalos), due to individual sensitivity to the drugs, environmental stimulation or inadequate IM administration through the dart. Some of the darts might have been SQ, hence interfering with the appropriate absorption of the drugs by the organism by slowing down the induction and decreasing the depth of the anesthesia. For these reasons, although a second dart dose was required, the immobilizations of 3 African buffalos, 2 roan antelopes and 2 lionesses were not classified as unsuccessful because the issue was easily identified and corrected without any associated complications. With that in mind, there were 176 cases from the 184 chemical immobilizations classified as 'well succeeded' (95.65%) and only 8 (4.35%) 'not succeeded' (individuals who required a second darting plus several top-up administrations or individuals that died during the procedures).

Most authors of scientific publications describe the doses of the drugs for ungulates as a net dosage for the species and only specify the dose according to the sex/approximate age of the animals (e.g. males, females, juveniles, calves). The doses for ungulates are not measured according to body weight, like, for example, with carnivores (Miller & Fowler, 2015; West, Heard & Caulkett, 2014; Kock & Burroughs, 2012 – Appendix 2. Table 20-34). Therefore the results presented on Chapter 3 tables for ungulates are also described as net doses not weight dependent.

In certain cases, such as the African buffalos, the protocol performed was based entirely on the experience of the veterinarian in charge and, as far as the author of this thesis is aware, the combination of etorphine with thiafentanil is not referred to in the literature for this particular species. The practitioner's years of experience allowed an improvement on the doses applied to the individuals of this species. The choice of the dart gun device (*Pneu-Dart* type C) was also based on the experience of the operator, plus the environmental conditions in the field. The characteristics of the darts were based on the volume of the drugs and thickness of the animal's skin (IM injection is preferable). WB needles allowed the dart to stay in place to facilitate the administration of the total volume of drugs.

The administration of medetomidine on some bulls and cows is associated not only with the size of the animal but also with the difficult conditions of the field (mountains, water courses, thick bush) which required a faster immobilization of the animals to avoid future complications after darting. However, the administration of atipazemole IM is always recommended to reverse the effects of medetomidine, which can also be used as a top-up drug when the animal does not show adequate signs of sedation. Midazolam, rather than medetomidine, was also often used to increase the muscle relaxation and improve the immobilization of buffalos. This particular drug is the top-up drug of choice by the veterinarian in charge of these procedures instead of ketamine, which has been commonly used in the past and well referred to in publications (Wolfe, 2015; Ball & Hormeyr, 2014; Lamont & Grimm, 2014; Burroughs et al., 2012b). As a dissociative agent, ketamine does not have any reversal drug and it presents more side-effects than midazolam, which can last up to 2-3 hours after administration and is excessively long for most of the usual procedures performed (Lamont & Grimm, 2014; Plumb, 2008; Caulkett & Arnemo, 2007).

The immobilizations of sable and roan antelopes also required administration of midazolam through the auricular vein to improve muscle relaxation. Medetomidine, also used in these antelope species, is referenced by a few practitioners (Wolfe, 2015; Ball & Hormeyr, 2014; Citino, Bush, Grobler & Lance, 2001), and it was always added to the darting cocktail for the roan antelopes. The characteristics of the darts used were also based on veterinarian experience. Larger antelope species required longer lengths when compared to smaller antelopes immobilized, such as bonteboks or springboks, which are targetable with darts of small volumes and lengths. It would be possible to use WB instead of the GC darts on the bontebok, but, for the volume of drugs required, the only available darts at the time were the GC.

The 2 male golden wildebeests restrained were administered with LAN – zuclopenthixol – to improve the tranquilization during the transportation of the animals. After the effect of the azaperone subsided, the animals required additional tranquilizers to improve not only the transportation but also the adaptation to the new environment (Wolfe, 2015; Read et al., 2000).

The plains zebras were successfully immobilized with etorphine (and azaperone), which is the best opioid for this particular species according to the references from the main wildlife veterinarians (Janssen & Allen, 2015; West et al., 2014; Walzer, 2014; Kock & Burroughs, 2012).

Although the immobilization of the ram bushbuck was an emergency, the protocol chosen (thiafentanil and medetomidine), and the dart gun/darts (*Pneu Dart* type C, 1cc 1''GC) worked successfully and restraint was accomplished. However, to guarantee a more efficient drug delivery to the animal, a WB dart would have been a safer choice. However, at the time a dart with a low volume, short length and WB was not available.

The delivery system manufacturer used for the darting was mainly the *Pneu Dart*, with some variations between type P and type C. Type C was used almost exclusively for African buffalos because of the veterinarian's experience with that type of gun/darts in that particular species, or for animals shot from a helicopter, such as the giraffes or the free-ranging bull elephant. The other manufacturer, *Dan Inject,* was only used for the immobilization of elephants in controlled environments to perform a standing sedation of the individuals. They were darted from the ground and, because of the quieter sound of the *Dan Inject* gun, in comparison to the *Pneu Dart*, the animals were less stressed after the shooting, allowing a smoother standing sedation.

Results obtained from the different immobilizations performed during the internship show that only 3 of the 245 animals immobilized died: 1 female springbok (which ran into a lake and drowned during the excitement phase); 1 female giraffe (which fell into a mud pit and was euthanized after a few hours of unsuccessful attempts by personnel to pull it out, and after showing signs of inappropriate muscular function on its posterior legs); and 1 female white rhino (which was extremely sensitive to the immobilization drugs). This female rhino was accurately darted with a rigorous cocktail of drugs because of historical problems with anesthetic drugs in its ancestors. However, the first dart was not efficient and the drugs were not administered on the animal. In order to immobilize the female to perform horn trimming, a preventive method for poaching issues in the country, a second dart with the same combination of drugs and doses was fired. Unfortunately, and despite all attempts to reverse the anesthesia, the rhino died.

The female giraffe was not using her posterior legs and, although no bone fracture was suspected, the muscles were too weak for the giraffe to hoist herself out of the pit. The most probable diagnostic was myopathy after a few hours of constant attempts by the team to pull the giraffe out. The entire capture procedure went as normal and the problem was a difficult one to prevent. One conclusion drawn from this incident is that it is always good practice to have a helicopter pilot with experience on hand to help prevent situations such as this. The other clinical cases with giraffes went faultlessly. The darts were only filled with opioids in high doses to guarantee a faster sedation of the animals and avoiding long excitement phases. Additionally, the darts had hyaluronidase powder to improve the fast action of the opioids in the organism (Morkel, 1993; Morkel, 1992). These opioids must be quickly reversed with the antidotes (naltrexone) immediately after the giraffe falls to the ground, otherwise high concentrations of opioids can be lethal to these animals (Bertelsen, 2015; Citino & Bush, 2014; Burroughs et al., 2012b). Then, the procedure must be carried out with the animal still on the ground and physically controlled by the team, which, in our study, had to walk the giraffes directly to the transport vehicle (Figure 14).

Figure 14 – Female giraffe guided by rangers into a transportation vehicle, after the reversal agent has been administered (Original).

Potential complications during wildlife immobilizations might warrant a previous discussion between the veterinarian in charge and the personnel involved in the restraint, to assess whether deaths can be expected during the operation. Based on the fact that 10% is an acceptable rate when the practice is being performed in species without a conservation value concern, a lower percentage of losses must be expected for threatened species (Hernandez, 2014). Out of all 245 cases, only 3 (1.2%) resulted in the loss of individuals. Two of those individuals were classified as LC species and the white rhino as NT, which means that they are not threatened species according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org). Thus the percentage lower than 10% referred by Hernandez (2014) can be compared to the 1.2% of losses in our study. These results confirm the efficiency and professionalism during the operations. However, comparing the chemical restraints performed in the 245 clinical cases examined, 184 were chemical immobilizations, 3 of which resulted in deaths. This equals 1.6%, which is still a much lower percentage, reinforcing the high standards of the methods, protocols and doses chosen.

Besides the 3 death cases, an attempt to immobilize a free-ranging bull elephant for a standing sedation was also unsuccessful although it was the first standing sedation attempt on a free-ranging bull African elephant. In this case, the drug cocktail in the dart was not strong enough to restrain the bull and a second dart with a new cocktail of drugs was given based on drugs and respective doses for a free-ranging bull knockdown immobilization. For the standing sedation of bull elephants in a controlled environment, one of the immobilizations required an additional administration of drugs to improve the depth of the anesthesia during the restraint as no pure opioids like etorphine were administered.

In carnivores, most of the procedures were well succeeded, except the male leopard, the male cheetah and one of the female painted dogs. The black leopard case was considered unsuccessful due to the second darting and the various top-ups required before performing surgery. All the surgeries in felids (hemi-hysterectomies, ophthalmological

procedures, castrations and epididymectomies) were performed without severe complications. The only requirement was the administration of medetomidine to improve the depth of the anesthesia, which was always reversed with atipamezole, and/or TZ, as is recommended by wildlife practitioners (Lamberski, 2015; Ramsay, 2014; Burroughs et al., 2012b; Jacquier, Aarhaug, Arnemo, Bauer & Enriquez, 2006; Fahlman et al., 2005). No further problems arose after these procedures, not even in the free-ranging individuals released into a non-controlled environment. The castration was performed on a male black leopard and the epididymectomies were carried out on male African lions because of the inherent difficulties of performing a vasectomy. The hemi-hysterectomies, performed on the lionesses (Figure 15 and 16) allow the free-ranging females to give birth every year, which is a very attractive feature for tourists and a significant help to the worldwide survival of the species without overloading a wildlife eco-reserve with too many predators. In the controlled facilities, the reproductive issues were managed with SQ GnRH implants.

Figure 15 and 16 – African lioness being prepared for surgery (left) and hemi-hysterectomy (right) in Gondwana Game Reserve (Original).

Although the male cheetah and one of the female painted dogs immobilized were classified as ‘not succeeded’ cases because of the requirement of a second dart dose plus the administration of top-up drugs to improve the depth of anesthesia (possible incorrect evaluation of body weight), all re-locations were well accomplished. The individuals were supplemented and regularly checked during transportation to prevent severe dehydration and ensure their welfare. Given that these two species in particular are classified as *Vulnerable* (Cheetah) and *Endangered* (Painted dog), they were among the threatened individuals examined during the internship. The role of the veterinarian with individuals of these species is vital, particularly during outbreaks of diseases like rabies, which can have a huge impact on the free-ranging populations (Padilla & Hilton, 2015; Lamberski, 2015; Ramsay, 2014; Good, Marobela, & Houser, 2005; Turnbull et al., 2004; Hofmeyr, Bingham, Lane, Ide & Nel, 2000; Jäger, Booker, & Hübschle, 1990).

CHAPTER 5. CONCLUSION

As protocols for each medical procedure can be influenced by SECONDS (Blumstein et al., 2015; Wolfe, 2015; Hernandez, 2014; Pas, 2014; Paterson, 2014; Hofmeyr et al., 2012; Sanchez, 2011), the experience of the veterinarian in charge is a crucial factor in choosing the most appropriate combination of drugs and respective doses. It is also vital to deciding the best delivery system according to the situation and the most efficient reversal, preventing possible complications during the restraint and turning it into an efficient and safe performance.

The majority of the immobilizations performed were well accomplished for both ungulates and carnivores and did not require any additional drugs administered to the animals. Percentage-wise the losses (1.2%) are well below the acceptable 10%, and no *Threatened* species were lost. However, besides the positive balance of the restraints

performed, this was only possible thanks to the experience of the veterinarian in charge and the experienced personnel involved.

There are many obstacles to this particular area of veterinary medicine and the practitioners must have the necessary qualifications and experience to be able to coordinate the team involved in field procedures and predict any possible complications during the restraint event. The success of a clinical case relies on the fore planning of the procedure and the prediction of potential problems. This is a transversal situation in all fields of veterinary practice. However, in wildlife medicine, practitioners are often examining species on the brink of extinction and dealing with conservational status concerns. Failures or mistakes that can be prevented are inadmissible.

The chemical restraint protocols described by the different authors Miller & Fowler, 2015; West et al., 2014; Kock & Burroughs, 2012 (see Appendix 2. Tables 20-34) and the ones performed during the internship present several unconformities in terms of the cocktails used for immobilization, doses and reversal options. The lack of information about the characteristics of the darts and respective dart guns that can be used to capture the different species is also an important aspect to be taken into consideration, especially given the versatility of procedures and situations that can arise, and which the authors rarely describe in scientific publications.

The veterinarian's experience in SECONDS dictates the different approaches that will ultimately contribute to the constant evolution of veterinary practice. Most of the academic compilations available do not clarify the factors that might influence the procedures (SECONDS) nor provide a protocol or specific doses for the species to which they are referring.

It is also important to note that some of the cocktail options presented in publications are only based on one or a few immobilizations and, most of the time, involve the same drug combinations and doses for free-ranging individuals as for individuals in a controlled facility (e.g. zoo) (Miller & Fowler, 2015; West et al., 2014).

It is vital that the experience of the different wildlife practitioners becomes standardized and is shared among veterinarians worldwide. Even data information about mistakes and cases that failed, complications observed in unusual situations and erroneous restraints performed in different scenarios, are fundamental to avoid as many losses as possible in the future. All relevant information collected must be reported.

Various global threats compromise the perspectives of survival for certain African species. However, it is important that all veterinarians who intervene in free-ranging animals or in a zoological environment know exactly what they are dealing with, without contributing towards the deterioration of the condition of the species worldwide. This new role of the

wildlife practitioner has already been referred to by Deem, 2015; Mazet, Hamilton & Dierauf, 2006 and Kock, Soorae & Mohammed, 2007, which define the jobs of future wildlife veterinarians as being consistent not only with medicine but also with conservation. New data information about successful or unsuccessful performances (in such as anesthetic protocols used during the internship, surgeries performed and even restraint techniques executed for certain species) should also be taken into account and published to promote the growth of knowledge in this branch of veterinary medicine. Constant improvement is the key that allows a more efficient and safer medical care for every single individual; whether free-ranging or in a zoo/controlled environment and, ultimately, will benefit the population worldwide.

Personally, I must reinforce the importance of the internship from which this thesis originated. This work allowed me to understand how challenging the veterinary practice can be, particularly working with wildlife and all the constraints of practicing it among free-ranging populations in remote areas with unpredictable logistic complications and environmental obstacles. It was not only vital for my academic education but was also an enriching experience and a privilege to be able to help what are among the most incredible species in the world, some of them almost on the brink of extinction.

CHAPTER 6.

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APPENDIX 1.

Table 19 – Characteristics of the dart needles for the most commonly immobilized African species, based on personal experience plus Dr Brendan Tindall's years of experience.

SPECIES		NEEDLE
Large herbivores	Rhino Elephant	60mm collared/barbed; 2-3mm diameter
	Buffalo, Giraffe, Eland	40-60mm collared/barbed; 2mm diameter
	Antelope (Sable, Roan, Gemsbok, Wildebeest, Kudu), Zebra	30-40 mm barbed; 2 mm diameter
Medium herbivores	Medium Antelope (Impala, Springbok)	20-30mm collared/barbed; 1-2mm
Small herbivores	Small antelopes (Duiker, Steenbok)	20mm collared/barbed; 1-1.5mm diameter
Large carnivores	Lion, Leopard, Cheetah, Painted dog	25-35mm barbed; 1.5mm diameter

APPENDIX 2.

The next sequence of tables shows the different protocols referred to by the main wildlife practitioners Kock & Burroughs, 2012; West et al., 2014 and Miller & Fowler, 2015 for the chemically immobilized species during the internship. The weight of the species was established according to the references from Miller & Fowler (2015).

Most of the doses published for ungulates are net doses in mg and not calculated on the weight of the animal (mg/kg). This is because of the difficulties in evaluating the weight of the animals, which can only really be achieved in a zoo environment, and environmental conditions that might influence the procedure and, consequently, the doses. Bearing in mind that most of the clinical cases in Chapter 3 were performed on free-ranging ungulates, the doses were also described as a net dose for the various species.

Table 20 – Different anesthetic protocols for the African buffalo.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Bull (300-900kg)	(boma) Etorphine:5-8mg+Azaperone:40-80mg or Xylazine:10mg/ (free-ranging) Etorphine:8-10mg + Azaperone:80-150mg or Xylazine:70-90mg/Thiafentanil:6-10mg + Azaperone:50-100mg	Xylazine: 0.2-0.4mg/kg / Etorphine:0.012mg/kg + Xylazine:0.1mg/kg / Etorphine:0.005-0.007mg/kg + Acepromazine:0.02-0.03mg/kg + Xylazine:0.08-0.18mg/kg / Etorphine:0.015mg/kg+Azaperone:0.15mg/kg / Carfentanil:0.006-0.008mg/kg /Carfentanil:0.002-0.005mg/kg + Xylazine:0.028-0.05mg/kg /Thiafentanil:0.007-0.014mg/kg + Azaperone:0.06-0.07mg/kg	Carfentanil: 0.005mg + Xylazine:0.05mg/kg / Etorphine:0.015mg/kg + Xylazine:0.1-0.15mg/kg / Thiafentanil:0.01-0.025mg/kg + Medetomidine:0.05-0.1mg/kg
Cow (300-900kg)	(boma) Etorphine:5-8mg+ Azaperone:40-80mg or Xylazine:10mg/ (free-ranging)Etorphine:6-10mg + Azaperone:80-100mg or Xylazine:40-60mg/Thiafentanil:6-8mg + Azaperone:50-100mg		
Reversal	Diprenorphine:16-25mg(bull)14-20mg(cow) if free-ranging or 15-20mg if in bomas / Naltrexone 15xThiafentanil or 80-150mg(bull) 180-200mg(cow) if Thiafentanil	Diprenorphine:0.01-0.03mg/Kg (Etorphine) or Naltrexone: 0.07-0.8mg/Kg (Carfentanil or Thiafentanil)	Naltrexone 50xEtorphine IV or 30xThiafentanil IV or 100xCarfentanil IM or IV/ Atipamezole 5xMedetomidine or 0.1mg/kg (Xylazine) IM

Table 21 – Different anesthetic protocols for the Sable antelope.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Bull (190-300Kg)	Etorphine:6-8mg or Thiafentanil:6-8mg + Azaperone:80-100mg/ Medetomidine:2-3mg/Detomidine:2-3mg/ Xylazine:10-15mg	Etorphine:4-5mg+ Azaperone:100mg/ Carfentanil:4.6mg + Xylazine:45mg	Carfentanil:0.015-0.02mg/kg + Xylazine:0.15-0.2mg/kg/ Etorphine:0.015-0.025mg/kg + Xylazine:0.1-0.2mg/kg + Ketamine:0.15-0.2mg/kg / Thiafentanil:0.03mg/kg + Xylazine:0.1-0.2mg/kg
Cow (190-300Kg)	Etorphine:4-5mg or Thiafentanil:4-6mg + Azaperone:80-100mg/ Medetomidine:2-3mg/Detomidine:2-3mg/ Xylazine:10-15mg	Etorphine:3-4mg+ Azaperone:100mg/ Carfentanil:2.9mg + Xylazine:28mg	
Reversal	Naltrexone:90-120mg(bull), 60-90mg (cow) or Diprenorphine:15-20mg(bull), 10-12mg(cow) (if Etorphine) + Atipamezole 5xMedetomidine or Detomidine + Yohimbine: 1mL/50Kg	Diprenorphine 2.5x Etorphine or Naltrexone 15x Etorphine	Naltrexone 50xEtorphine IV or 30xThiafentanil IV or 100xCarfentanil IM or IV/ Atipamezole 5xMedetomidine or 0.1mg/kg (Xylazine) IM

Table 22 – Different anesthetic protocols for the Roan antelope.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Bull (190-310Kg)	Thiafentanil:6-10mg/ Etorphine:6-8mg +Azaperonel:60-100mg/ Medetomidine:5-6mg/ Detomidine:3-5mg/ Xylazine:20-30mg	Etorphine:4-5mg+ Azaperone:100mg/ Carfentanil:5.6mg + Xylazine:56mg	Carfentanil:0.015-0.02mg/kg + Xylazine:0.15-0.2mg/kg/ Etorphine:0.025mg/kg + Xylazine:0.15-0.25mg/kg / Thiafentanil:0.01-0.02mg/kg + Medetomidine:0.005-0.006mg/kg + + Ketamine:0.3-0.6mg/kg
Cow (190-300Kg)	Thiafentanil:6-8mg/ Etorphine:5-7mg +Azaperonel:50-80mg/ Medetomidine:3-5mg/ Detomidine:3-5mg/ Xylazine:20-30mg	Etorphine:3-4mg+ Azaperone:100mg/ Carfentanil:4.2mg + Xylazine:42mg	
Reversal	Naltrexone:75-150mg(bull), 90-120mg (cow), 60-125mg (juvenile), 45mg (calf) or Diprenorphine:20mg(bull), 13-18mg(cow) (if Etorphine) + Atipamezole 5xMedetomidine or Detomidine + Yohimbine: 1mL/50Kg	Diprenorphine 2.5x Etorphine or Naltrexone 15x Etorphine	Naltrexone 50xEtorphine IV or 30xThiafentanil IV or 100xCarfentanil IM or IV/ Atipamezole 5xMedetomidine or 0.1mg/kg (Xylazine) IM

Table 23 – Different anesthetic protocols for the Springbok.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Ram (30-45Kg)	Thiafentanil:0.5-1mg/ Etorphine: 0.5mg/ Fentanyl:10-15mg + Medetomidine:1mg/ Azaperone:10-20mg/ Xylazine:2-5mg	No data	Carfentanil:0.03mg/kg / Etorphine:0.05-0.1mg/kg + Xylazina:0.15-0.25mg/kg / Xylazine:0.5mg/kg + Ketamine:9mg/kg
Ewe (30-45Kg)			
Reversal	Naltrexone 10-15mg or Diprenorphine:2-3mg (if Etorphine), 2.5mg (if Fentanyl)	No data	Naltrexone 50xEtorphine IV or 30xThiafentanil IV or 100xCarfentanil IM or IV/ Atipamezole 0.1mg/kg (Xylazine) IM

Table 24 – Different anesthetic protocols for the Bontebok.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Ram (55-80Kg)	Thiafentanil:3mg+ Azaperone:40mg or Xylazine:5mg or Detomidine:3-5mg /Etorphine:3mg+Azaperone:40mg	No data	Carfentanil:0.015-0.025mg/kg + Xylazine:0.2-0.35mg/kg + Ketamine:1.5-2.5mg / Etorphine:0.02-0.025mg/kg + Xylazine:0.2-0.3mg/kg + Ketamine: 0.2-0.3mg/kg / Thiafentani:0.03mg/kg + Azaperone:0.5mg/kg / Medetomidine:0.05-0.09mg/kg + Ketamine:1-1.3mg/kg
Ewe (55-80Kg)			
Reversal	Naltrexone 45-60mg or Diprenorphine 6-9mg	No data	Naltrexone 50xEtorphine IV or 30xThiafentanil IV or 100xCarfentanil IM or IV/ Atipamezole 5xMedetomidine or 0.1mg/kg (Xylazine) IM

Table 25 – Different anesthetic protocols for the Giraffe.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Bull (850-1950Kg)	Etorphine:12-16mg / Thiafentanil:14-20mg *	Etorphine:8-15mg / Thiafentanil:8-16mg / Etorphine:4mg+Thiafentanil:8-16mg	Etorphine or Thiafentanil up to 18mg/ Thiafentanil up to 9mg + Etorphine up to 9mg / Thiafentanil:0.006-0.01mg/kg + Medetomidine:0.01-0.014mg/kg + Ketamine:0.5mg/kg
Cow (700-1200Kg)	Etorphine:10-12mg / Thiafentanil:12-14mg *		Etorphine or Thiafentanil 14-15mg/ Thiafentanil:7-7.5mg + Etorphine: 7-7.5mg/ Thiafentanil:5-8.3mg + Medetomidine:8.3-11.6mg+ Ketamine:415mg
Reversal	Naltrexone10x opioid or Diprenorphine 2.5x Etorphine	Naltrexone 30-100x Etorphine or Diprenorphine 2xEtorphine/ Naltrexone 30xThiafentanil	Naltrexone 0.3-0.4mg/Kg (if Thiafentanil and Etorphine), 0.2-0.3mg/Kg + Atipamezole 0.05mg/Kg

Table 26 – Different anesthetic protocols for a standing sedation of African elephants in controlled environments.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Bull (4000-7000Kg)	No data	Azaperone: 0.06-0.15mg/kg / Butorphanol: 0.12mg/kg + Xylazine: 0.2-0.3mg/kg / Xylazine: 0.11-0.55mg/kg / Detomidine: 0.014-0.0162mg/kg + Butorphanol: 0.014-0.0162mg/kg	Butorphanol:0.013-0.02mg/kg + Detomidine: 0.013-0.02mg/kg
Reversal	No data	Yohimbine: 0.5xXylazine Atipamezole:0.1xXylazine	Naltrexone 50-100x opioid/ 2-3.5mg/Kg + Atipamezole 0.1-0.16mg/Kg

Note: There is no published anesthetic protocol for a standing sedation on a free-ranging African elephant.

Table 27 – Different anesthetic protocols for the White rhino.

	Kock & Burroughs, 2012	West et al., 2014	Miller & Fowler, 2015
Cow (1800-2200Kg)	Etorphine:2-4mg + Butorphanol: 30-80mg + Azaperone:40-60mg or Detomidine:2-4mg	Etorphine:2-3.5mg+Butorphanol:40-90mg+Midazolam:25-50mg / Etorphine:3-4.5mg+Azaperone:40-60mg+Detomidine:10-20mg	Carfentanil:1.2mg / Etorphine:2-3mg + Azaperona:20-40mg / Butorphanol:120-150mg + Medetomidine:5-7mg / Butorphanol:70-120mg + Azaperone:100-160mg
Reversal	Naltrexone:40-80mg or Diprenorphine: 6-12mg	Naltrexone 40x Etorphine IV or Diprenorphine 2-2.5xEtorphine IV	Naltrexone 40-100mg/mg Etorphine or 1-2.5mg/mg Butorphanol (+ Atipamezole 5x Medetomidine)

Table 28 – Different anesthetic protocols for the Golden wildebeest (Blue wildebeest).

	Kock & Burroughs, 2012	West et al., 2014	Miller & Fowler, 2015
Bull (110-180Kg)	Thiafentanil:4-6mg or Etorphine: 5-7mg + Azaperone:100mg or Medetomidine: 5-6mg or Detomidine:10 mg or Xylazine: 20-40mg	Etorphine:4-5mg + Azaperone:100mg	Carfentanil:0.008mg/kg + Xylazine:0.08mg/kg / Thiafentanil:0.03mg/kg + Xylazine:0.1mg/kg
Cow (110-180Kg)	Thiafentanil:3-5mg or Etorphine: 3-5mg + Azaperone:80mg or Medetomidine: 5-6mg or Detomidine:10 mg or Xylazine: 5-10mg	Etorphine:3-4mg + Azaperone:100mg	
Reversal	Bull: Naltrexone 55-90mg or Diprenorphine:12-17mg; Cowl: Naltrexone 45-75mg or Diprenorphine:10-13mg + Atipamezole 5x Medetomidine/Detomidine or Yohimbine: 1ml/50kg	Diprenorphine 2.5x Etorphine or Naltrexone 15x Etorphine	Naltrexone 30xThiafentanil IV or 100xCarfentanil IM or IV

Table 29 – Different anesthetic protocols for the Plains zebra.

	Kock & Burroughs, 2012	West et al., 2014	Miller & Fowler, 2015
Stallion (175-275Kg)	Etorphine: 4-7mg + Azaperone:40-60mg or Medetomidine: 5mg or Detomidine:5-10 mg or Xylazine: 40-60mg	Etorphine:0.0085-0.01mg/kg + Acepromazine:0.035-0.04mg/kg / Tiletamine+Zolazepam:5.5-8.3mg/kg + Detomidine:0.06-0.08mg/kg	Detomidine: 0.1mg/kg + Butorphanol:0.13mg/kg
Mare (175-275Kg)	Etorphine: 4-6mg + Azaperone:40-60mg or Medetomidine: 5mg or Detomidine:5-10 mg or Xylazine: 40-60mg		
Reversal	Naltrexone 60-100mg or Diprenorphine 10-18mg (Stallion) or 10-15mg (Mare)	Diprenorphine 0.045mg/kg IV (for Etorphine)	Naltrexone: 0.1mg/kg + Atipamezole 2xDetomidine IV

Table 30 – Different anesthetic protocols for the Bushbuck.

	Kock & Burroughs, 2012	West et al., 2014	Miller & Fowler, 2015
Ram (40-50Kg)	Thiafentanil:3mg+ Azaperone:40mg or Xylazine:5mg or Detomidine:3-5mg /Etorphine:3mg+Azaperone:40mg	No data	No data
Reversal	Naltrexone 45-60mg or Diprenorphine 6-9mg	No data	No data

Table 31 – Different anesthetic protocols for the African lion.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Lion (120-250kg)	TZ:0.5mg/kg + Medetomidine: 0.03mg/kg / TZ:3-5mg/kg / Ketamine:5mg/kg+ Xylazine:0.5mg/kg / Ketamine:5mg/kg + Medetomidine:0.05mg/kg / Butorphanol:0.2-0.3mg/kg + Medetomidine:0.05mg/kg + Midazolam:0.15mg/kg	TZ: 0.6-1mg/kg + Medetomidine:0.015-0.025mg/kg / TZ: 4-6mg/kg/ Ketamine:7-10mg/kg+ Xylazine:1-4mg/kg / Ketamine:1.9-5.7mg/kg + Medetomidine:0.02-0.08mg/kg[a]	TZ:1.6-4.2mg/kg / Ketamine:3-10mg/kg+ Xylazine:0.3-1mg/kg / Ketamine:2-6mg/kg + Medetomidine:0.03-0.07mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg + Ketamine:1-2mg/kg
Lioness (120-250kg)	TZ:0.5mg/kg + Medetomidine: 0.03-0.05mg/kg / TZ *l*:3-5mg/kg / Ketamine:5mg/kg+ Xylazine:0.5mg/kg / Ketamine:5-7mg/kg + Medetomidine:0.03-0.05mg/kg / Butorphanol:0.2-0.3mg/kg + Medetomidine:0.05mg/kg + Midazolam:0.15mg/kg		
Reversal	Atipamezole 5xMedetomidine / Yohimbine:1mL/50Kg IV (if Xylazine) /Naltrexone:2xButorphanol	Atipamezole0.1-0.29mg/kg (if a) / Yohimbine 0.1mg/Kg IV if Xylazine	Atipamezole / Naltrexone / Yohimbine/ Flumazenil if need it (doses without data)

Table 32 – Different anesthetic protocols for the Cheetah.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Male (35-72Kg)	TZ:0.5mg/kg + Medetomidine: 0.03-0.05mg/kg / TZ:3-5mg/kg / Ketamine:3-5mg/kg+ Xylazine:0.5mg/kg / Ketamine:3-5mg/kg + Medetomidine:0.03mg/kg / Butorphanol:0.2mg/kg + Medetomidine:0.15mg/kg + Midazolam:0.03mg/kg / *Saffan*®:60-120mg (alphaxolone 9mg/mL + alphadolone:3mg/mL)	TZ: 1.6-7.8mg/kg / Medetomidine:0.035mg/kg+ Butorphanol:0.02mg/kg + Midazolam:0.15mg/kg[a] / *Saffan*®:5mL IV (alphaxolone 9mg/mL + alphadolone:3mg/mL) / Ketamine:1.57-2.5mg/kg + Medetomidine: 0.031-0.07mg/kg[b]	TZ:1.6-4.2mg/kg / Ketamine:3-10mg/kg+ Xylazine:0.3-1mg/kg / Ketamine:2-6mg/kg + Medetomidine:0.03-0.07mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg + Ketamine:1-2mg/kg
Female (35-72Kg)			
Reversal	Atipamezole 5xMedetomidine / Yohimbine:1mL/50Kg IV (if Xylazine) / Naltrexone 2xButorphanol	[a]Atipamezole:0.18mg/Kg + Flumazenil:0.006mg/Kg + Naltrexone: 0.25mg/Kg / [b]Atipamezole: 0.15-0.3mg/kg	Atipamezole / Naltrexone / Yohimbine/ Flumazenil if needed (doses without data)

Table 33 – Different anesthetic protocols for the Leopard.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Male (23-91Kg)	TZ:1-3mg/kg + Medetomidine: 0.05-0.08mg/kg / TZ:5-10mg/kg / Ketamine:8-10mg/kg+ Xylazine:1mg/kg / Ketamine:5-10mg/kg + Medetomidine:0.05-0.08mg/kg	Ketamine:5-10mg/kg + Xylazine:1-4mg/kg	TZ:1.6-4.2mg/kg / Ketamine:3-10mg/kg+ Xylazine:0.3-1mg/kg / Ketamine:2-6mg/kg + Medetomidine:0.03-0.07mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg / Butorphanol:0.003-0.04mg/kg + Medetomidine:0.1-0.4mg/kg + Midazolam:0.1-0.3mg/kg + Ketamine:1-2mg/kg
Reversal	Atipamezole 5xMedetomidine / Yohimbine:1mL/50Kg IV	No data	Atipamezole / Naltrexone / Yohimbine/ Flumazenil if needed (doses without data)

Table 34 – Different anesthetic protocols for the African wild dog.

	Kock & Burroughs, 2012	**West et al., 2014**	**Miller & Fowler, 2015**
Male (19-35Kg)	TZ:0.5mg/kg + Medetomidine: 0.03-0.05mg/kg or Xylazine 0.5mg/kg / TZ:3-5mg/kg / Ketamine:5mg/kg+ Medetomidine:0.1mg/kg / Fentanyl:2.5mg + Xylazine:0,5mg/kg / Butorphanol:0.15mg/kg + Medetomidine: 0.05mg/kg	Ketamine:3-5mg/kg + Medetomidine:0.005-0.1mg/kg [a]/ Medetomidine:0.045mg/kg + Butorphanol:0.24mg/kg + Midazolam:0.3mg/kg / Xylazine: 0.7-1.1mg/kg + Fentanyl: 0.1mg/kg + bolus o 10mg of Xylazine and 0.5mg of Fentanyl / Ketamine: 1.6mg/kg + Xylazine: 2.2mg/kg / TZ:1-4mg/kg	Medetomidine: 0.04-0.06mg/kg + Butorphanol: 0.18-0.3mg/kg + Midazolam: 0.18-0.4mg/kg / Ketamine:1.5-5mg/kg + Medetomidine:0.04-0.1mg/kg / TZ:1-4 mg/kg
Female (19-35Kg)			
Reversal	Atipamezole 5xMedetomidine; Naltrexone 2xButorphanol; Yohimbine (no dose available) if Xylazine	Atipamezole:0.1mg/Kg if a, 3mg/Kg if b (plus Naltrexone:10mg + Flumazenil:0.2mg); Yohimbine:0.125mg/Kg + Naloxone:0.04mg/Kg if c; Yohimbine:0.2mg/Kg if d	No data